DEVELOPMENT OF MODELS TO STUDY DENTAL EROSION

Thesis submitted in accordance with the requirements of the

University of Liverpool for the degree of Doctor in Philosophy

By

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November 2007

THE FACULTY OF MEDICINE
(School of Dental Sciences)

UNIVERSITY OF
LIVERPOOL

Contents

Index of Figures

Index of Tables

Abbreviations

ΔQ	Integrated value of ΔF over lesion area in mm^2/pixel
ΔZ	Integral value representing mineral loss or gain
ΔZs	Integral value representing mineral loss or gain of only surface
µm	Micrometer
2D	Two dimensional
3D	Tree dimensional
adj.R^2	Adjusted Person correlation coefficient squared
ANOVA	Analysis of variance
CCD	Charge-coupled device
g/L	Gram per litre
kV	Kilovolt
ld	Lesion depth
ldc	Lesion depth of crater
lds	Lesion depth of only surface
mA	Milliampere
mg/L	Milligram per litre
mm^2	Millimetre squared
mmol/L	Millimole per litre
nm	Nanometre
OCT	Optical coherence tomography
ppm	Parts per million
QLF	Quantitative Light-induced Fluorescence
rpm	Revolutions per minute
SD	Standard deviation
SPSS	Statistical Package for the Social Sciences
TMR	Transverse Microradiography
vol	Volume
w/v	weight/volume

Acknowledgements

First, I wish to thank my supervisors Professor Susan M. Higham and Dr. Bennett T. Amaechi. I am very grateful to Sue for her continuous and tremendous support and understanding throughout all my studies. I am personally indebted to Ben for teaching me all techniques and guiding me in the planning of my experiments.

I would like to acknowledge Professor Neil Pender for his constant support and encouragement throughout my PhD studies.

I would like to express my gratitude to Dr. Emmanuel Agalamanyi. Without his caring assistance and valuable advice I could not have succeed in completing of my laboratory work.

I gratefully acknowledge the generous support of Dr. Phil Smith, who spared much of his own precious time offering valuable help with English grammar.

Special thanks go to Dr. Adejumoke Adeyemi, my friend Jumoke, for being so kind, helpful, understanding and supportive, without whose encouragement I could not have finished writing my thesis.

Grateful thanks are also due to my colleagues and all staff of The Edwards Building who appreciably contributed to my studies at The University of Liverpool, especially to Dr. John Stanbury, Janette Chesters, Jim Hibbard, Lee Cooper.

I am particularly grateful to the late Professor Graham Embery for his support during my first two years in Great Britain, both research wise and personally.

I am appreciative of The University of Liverpool and especially to School of Dentistry for the financial support for my studies. In addition, I sincerely acknowledge the importance of 'Universities UK' for granting me with an 'ORS award', which led to a significant alleviation in financial burden of my PhD studies. I am also grateful to The Procter & Gamble Company, who provided financial support for the studies reported in Chapter 5.

Finally, I would like to thank my family, my wife Galya for her love, support, understanding and healthy criticism, and my daughter Ryta for being such a nice and caring child, and, of course, to my mum, my sister and other relatives and friends living far away in Belarus for their part in providing unconditional support and encouragement to pursue my interests during all the good times and the bad.

Declaration

This thesis is the result of my own work. The material contained in the thesis has not been presented, nor is currently being presented, either wholly or in part for any other degree or other qualification.

The research work was carried out in the Department of Dental Sciences, University of Liverpool.

Gleb Komarov

Publications and Presentations Arising from Work in This Thesis

Komarov GN, Amaechi BT, Higham SM. The Remineralisation Potential of Artificial Saliva with Different Concentrations of Calcium on Eroded Lesion *in-vitro*. Caries Research, 2002; 36-3:181 (abstract 23).

Komarov GN, Amaechi BT, Higham SM. Remineralising Effect of Fluoride on Enamel Erosion *in vitro*. Journal of Dental Research, 2003; 82-spec:508 (abstract 263).

Komarov GN, Amaechi BT, Higham SM. Quantitative Light-Induced Fluorescence Correlated with Transverse Microradiography for Quantification of Dental Erosion *in vitro*. Caries Research, 2003; 37:313 (abstract 135).

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ABSTRACT

Epidemiological studies confirm that toothwear and especially dental erosion is a significant oral health problem in all age groups and that the prevalence is increasing. It is believed that the two major factors are responsible for the development of this condition and they are: dietary and gastric acids, frequently appearing in the human mouth causing distinctive lesions on tooth enamel, which in severe cases can lead to complete loss of enamel and dentine. The increased interest in studying dental erosion suggests further research is required to find suitable and reliable methods for detection and quantification of erosion *in vivo*, *in situ* and *in vitro* as well as to discover the most appropriate means of prevention of dental erosion and treatment of its early manifestations.

Transverse microradiography (TMR) has been a useful technique in erosion studies; however it is a destructive procedure and therefore neither suitable for *in vivo* studies nor for longitudinal monitoring of the progression of this dental disease. The aim of this thesis was to develop *in vitro* model systems, which may be used to investigate dental erosion, to use these model systems in the evaluation of a non-destructive and non-invasive technique Quantitative Light-induced Fluorescence (QLF) as a tool for quantifying early dental erosion. It was shown that QLF was able to identify enamel erosion caused by orange juice *in vitro* and may be helpful to longitudinally assess changes in mineral content of dental enamel following an erosive challenge on both human and bovine teeth.

It is well known from caries studies as well as from some erosion data that the presence of calcium, phosphate and fluoride ions can significantly increase the remineralisation process in early enamel lesions and in some cases even to reverse it. Using remineralising solutions containing these protective ingredients, for example in patients, suffering from frequent vomiting episodes, could be beneficial from the point of hardening tooth enamel softened by low-pH gastric content. It is very important to determine the most effective concentration of active ingredients in these remineralising solutions as relatively low amounts of calcium and fluoride could increase its remineralising potential. It was found that artificial solutions containing 250 ppm calcium in the form of calcium lactate and 500 ppm fluoride in the form of sodium fluoride exhibit protective and remineralising properties on human teeth with artificially produced dental erosive lesions *in vitro*.

The composition and properties of soft beverages are of great interest since dental erosion has been associated with their consumption. The erosive potential of dietary drinks varies and can be modified using additives such as minerals and sugar substitutes. It was shown that the addition of 400 ppm calcium in the form of calcium malate citrate significantly reduced the erosive potential of acidic (pH below 4) fruit drinks, when it was used in a cycling model to produce artificial erosive lesion on human teeth *in vitro*.

Chapter 1

LITERATURE REVIEW

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1.1 Introduction

In the past decades in most countries of Europe and North America, extensive research, the introduction of preventive public programs and schemes related to dental health have led to a decline in the prevalence of dental caries - the major problem of oral health (Murray, 1998). Whilst dental caries has declined a newer threat to the mineralised dental tissues has emerged, namely toothwear. Once a condition related to physiological wear and tear of the teeth affecting elderly individuals, toothwear has become recognised as a problem in much younger individuals including children (Milosevic *et al*, 1994; Shenkin *et al*, 2003; Wiegand *et al*, 2006). Toothwear is thought to result from the interplay of attrition, abrasion or erosion although it is easy to envisage that these three factors could co-exist or exert their effects to a different extent at different times. Although the problem of dental erosion has been known for many years, disagreement still exists on the aetiology of this rather common dental condition. Loss of superficial tooth tissue as a result of erosion is very often made worse by abrasion and/or attrition (Davis and Winter, 1980). In these cases it is very difficult to make a clear differentiation between these pathological conditions. Due to this reason some authors prefer to name the process of pathological tooth surface loss as toothwear in spite of the fact that in some cases the only or main causative factor could be well established. In this review, no preference will be

given to any of the terms as both of them clearly denote the nature of the disease and will be used equally.

In younger people most attention has focused on erosive wear (Shenkin *et al*, 2003; Wiegand *et al*, 2006). Having achieved promising results in the area of dental decay, researchers shifted their attention to the diagnosis and monitoring of toothwear *in vitro* and *in vivo*, especially erosion. Because toothwear causes changes to the mineralised dental tissues laboratory and clinical techniques initially designed for studying dental caries have been applied to the study of toothwear. The results of recent epidemiological studies (Deery *et al*, 2000) as well as obvious changes in human dietary habits all over the world, for example the consumption of fizzy and fruit drinks (British Soft Drink Association. Report of Seminar in Heidelberg, 1991), which may exhibit erosive potential, have raised interest in studying the nature of dental erosion, its treatment and possible prevention.

This review will discuss the nature, epidemiology and severity of toothwear, together with the causative and concomitant factors of dental erosion as well as the potential use of protective agents against this condition. A summary of the contemporary quantitative and qualitative techniques to study dental erosion will be presented together with aspects on its prevention.

1.2 Origin of dental erosion

Pindborg (1970) described erosion as loss of dental tissue by a chemical process that does not involve bacteria. No doubt this chemical process implicates the action of the acidic environment, which leads to the disc-shaped and shallow defects on the tooth enamel surface. It is necessary to distinguish pathological loss of tooth structure from physiological, when hard dental tissue is liable to normal wear, but the question "what rate of toothwear should be considered to be pathological?", is still unclear.

The pathophysiology of dental erosion is complex with a number of potentially important risk factors. Erosion can have extrinsic or intrinsic causes, acting alone or in combination (Järvinen *et al*, 1991). Extrinsic factors include acid containing foods and drinks, such as citrus and other fruits, carbonated and fruit beverages (Lussi *et al*, 1991), raw food diet (Ganss *et al*, 1999) medicines like vitamin C (Meurman and Murtomaa, 1986) and sport drinks (Birkhed, 1984; Milosevic *et al*, 1997). Another extrinsic cause of erosion is acidic contaminates in the working environment. Exposure to industrial acids in air was common in developed countries before adequate occupational health measures were accepted (ten Bruggen Cate, 1968). Nowadays that type of environmental dental erosion is only rarely found in cases such as poorly maintained chlorinated swimming pools (Centerwall *et al*, 1986). There is also evidence that professional wine

testing could lead to excessive toothwear (Wiktorsoon *et al*, 1997) as the pH of wine can be as low as 2.8 (Sorvari and Rytömaa, 1991).

Dental erosion can also be related to the following intrinsic factors such as gastro-oesophageal reflux (Barlett *et al*, 1996), anorexia nervosa, bulimia nervosa (Hellström, 1977) and rumination (Gilmour and Beckett, 1993) when highly acidic gastric contents appear in the mouth after frequent voluntary or symptomatic regurgitation or vomiting.

1.3 Epidemiology

Epidemiological studies confirm that dental erosion is a significant problem in oral health. Surveys showed that the prevalence of toothwear is very high in all age groups with a tendency to be on the increase (Jaeggi and Lussi, 2006). Due to the different indices and examination techniques used and various epidemiological methods applied, it is difficult to compare the results in these studies. For example, Järvinen *et al* (1991) in a case-control study revealed that among 100 adults randomly chosen from patients visiting dental practices only 5 had signs of dental erosion. In another survey 16% of examined adults had teeth with mild palatal erosion and up to 40% with occlusal erosion (Lussi *et al*, 1991). Controversial results have also been observed in children and adolescents. Egermark-Eriksson (1982) reported that about 14% of 15-year-olds had exposed dentine due to toothwear in incisors and the same problem was revealed in front teeth of 78% of 15-18- year-olds by Nilner (1981). Milosevic *et al* (1994) demonstrated the prevalence of toothwear on a cohort of 14-year-old children living in Liverpool. About 30% of examined subjects had lesions into dentine, mostly incisally. In another survey 41% and 37% of adolescents in the USA and the UK respectively had toothwear on labial and palatal surfaces of upper incisors (Deery *et al*, 2000). Almost 50% of children aged 4 showed some sign of erosion in a study reported by Millward *et al* (1994a).

Besides the variations reported in the data relating to the prevalence of dental erosion, or toothwear (as a more integrated term), in the human population, there is disagreement as to whether the location of this dental condition in the dental arch is related to causal factors. It is believed that dental erosion prevailed more on the labial surfaces of upper anterior teeth than on lingual or occlusal surfaces. The palatal erosion of upper anterior teeth was associated mainly with intrinsic causes (Hellström, 1977; Hurst *et al*, 1977; Eccles, 1978; Bartlett *et al*, 1996). Labial surfaces were most often reported as affected due to exposure to acids in food and beverages, while occlusal surfaces of molars were reported almost unaffected (Eccles and Jenkins, 1974). More recent studies have reported a different pattern. Milosevic *et al* (1994) revealed that incisal or occlusal wear into dentine was more common than lingually and that there was no exposed dentine on the buccal surfaces in examined 14-year-old children with a significantly higher prevalence of toothwear in males than in females. Asher and Read, (1987), Millward *et al* (1994b) and Järvinen *et al* (1992) examined children and adults and demonstrated that palatal erosion predominated. The latter study also revealed that the risk of palatal erosion was 1.9 times greater in the group with gastric aetiological factors than in the group with dietary causes but the distribution of labial and lingual erosion within the gastric group was similar. In 1991, Lussi *et al* in a population of Swiss adults found that occlusal and buccal tooth surfaces were more often affected than lingual. Good correlation between the lingual location of erosion with vomiting and buccal erosion with dietary habits were observed. In another survey on 14-

year-old London schoolchildren (Williams *et al*, 1999) labial erosion of incisors was more prevalent that that of the palatal location, however the correlation between risk factors and dental erosion was not observed. In cases of industrial origin of toothwear only surfaces of anterior teeth not protected by soft oral tissues revealed eroded lesions (ten Bruggen Cate, 1968). It would appear that the precise distribution of dental erosion and toothwear in humans remains variable but is related to the nature of the erosive challenge and would benefit from further investigation.

1.4 Causative and concomitant factors

1.4.1 Extrinsic factors

1.4.1.1 Dietary acids

As previously mentioned, dietary acids are probably playing the most important contributory role in the development of pathological toothwear. The composition and properties of soft beverages are of great interest since dental erosion has been associated with their consumption. A number of studies in the 1940's and 1950's mainly on rat models *in vivo,* demonstrated the potential of different organic and nonorganic acids present in drinks to cause dissolution of tooth mineral (McClure, 1943; Restarski *et al*, 1945a; Wynn and Haldi, 1948; McCay and Will, 1949; Miller, 1950; Holloway *et al*, 1958). Later with the development of more precise and accurate techniques for quantifying tooth surface loss (Hartles and Wagg, 1962; Davis and Winter, 1977; de Josselin de Jong *et al*, 1987; Sorvari and Kiviranta, 1988; Mistry and Grenby, 1993; Brunton and Hussain, 2001) instead of using the scoring method (Restarski *et al*, 1945b) several studies were conducted *in vitro* (Smith and Shaw, 1987; Rytömaa *et al*, 1988; Grobler *et al*, 1989, Lussi *et al*, 1995), *in situ* (Rugg-Gunn *et al*, 1998; Hughes *et al*, 1999a; Amaechi *et al*, 2000) as well as on rats *in vivo* (Hartles and Wagg, 1962; Sorvari *et al*, 1988).

Acids such as phosphoric, citric, malic and lactic acids were found solely or in combination to be the most aggressive corrosive agents in the composition of cola beverages, fruit juices and drinks (Restarski *et al*, 1945a; Rytömaa *et al*, 1988; Grando *et al*, 1996). Orange (Grenby *et al*, 1989; Mistry and Grenby, 1993) and apple (Wynn and Haldi, 1948; Lussi *et al*, 1995) juice products had the greatest erosive potential among the fruit drinks varieties. Some of the herbal teas, present on the market, were also found to be erosive (Brunton and Hussain, 2001; Phelan and Rees, 2003).

The pH as well as the buffering capacities of beverages is important in relation to their potential to dissolve the superficial layer of dental enamel (Grenby *et al*, 1989; Lussi *et al*, 1995; Hughes *et al*, 2000). This rather simple chemical process starts at a pH level of about 5.0 (Meurman *et al*, 1990) and becomes pronounced at 3.5 for orange juice, which is the most common representative of the dietary erosive agents (Grenby *et al*, 1989; Mistry and Grenby, 1993). As all drinks that produce erosion are unsaturated with respect to hydroxyapatite and fluorapatite (Larsen, 1975) the concentration of calcium, phosphates and fluoride are important factors that influence enamel dissolution during an erosive challenge (Reussner *et al*, 1975; Grobler and van der Horst, 1982; Lussi *et al*, 1993; Hughes *et al*, 2000). The alteration of the constituents of beverages in respect of calcium, phosphates as well as fluoride in order to reduce their damaging potential could be of value in prevention of dental erosion.

Early studies with acid containing food substances highlight the fact that the presence of sweetening carbohydrates included in some drinks may alter the degree of dental erosion (Restarski *et al*, 1945a; Holloway *et al*, 1958). This effect may be explained by the influence of fermentable acids present in these formulations altering the oral pH (Birkhed, 1984), but more recently this finding was not observed (Grenby *et al*, 1989; Meurman *et al*, 1990).

1.4.1.2 Medicinal acid containing products

There are reports that frequent use of some medicaments and other health products of an acidic nature such as ascorbic acid (Giunta, 1983), acetylsalicylic acid (Grace *et al*, 2004) and certain oral hygiene products (Rytömaa *et al*, 1989) could contribute to the prevalence of dental erosion in humans. Some *in vivo* and *in vitro* studies have clearly reported the erosive effect of different prescribed health related substances, which could lead to distinctive tooth surface loss especially if their usage is abused or prolonged.

1.4.1.3 Behavioural factors

Some behavioural factors may also play a role in the development of dental erosion. A lifestyle that involves dieting, increased consumption of acidic fruits and vegetables; a high intake of acidic beverages and regular intense sporting activities are thought to lead to a higher risk of erosion (Zero, 1996).

1.4.2 Intrinsic acids

The relatively high prevalence of eating disorders (bulimia nervosa, anorexia nervosa) and some gastric dysfunctions such as gastroesophageal reflux disease correspond to the relevant dental problems (Hellström, 1977; Meurman *et al*, 1994) thus leaving these pathological conditions together with dietary habits among the major causal factors of dental erosion. The frequent appearance in the oral cavity of gastric acids with pH values usually below 2.0 – far below the 'critical pH' for the enamel hydroxyapatite, puts at high risk the integrity of dental hard tissue. Although patients with eating and gastric disorders have significantly more abnormal toothwear than healthy individuals, the correlation between the levels of erosion and frequency and the duration of vomiting is not clear, suggesting a complicated interrelation of causal and concomitant factors of dental tissue loss (Milosevic and Slade, 1989; Robb *et al*, 1995).

The erosive challenge on dental enamel is highly aggravated by the reduction of the salivary secretion due to atrophic processes in salivary glands in anorexic patients (Hellström, 1977), general dehydration following misuse of diuretics and laxatives and by deliberate vomiting (Clark, 1985), drug-therapy induced xerostomia (Meurman *et al*, 1994) and the significant increase of intake of acidic beverages due to thirst.

Oral hygiene procedures such as toothbrushing following vomiting episodes may also exacerbate the severity of dental erosion (Clark, 1985) due to

the abrasive effect of the toothbrush and toothpaste on the softened enamel produced by acids. However the relationship between the level of toothwear in patients with vomiting episodes and frequency of post-vomiting oral hygiene was not established (Milosevic and Slade, 1989; Robb *et al*, 1995) possibly because of the relatively small number of patients, which were used in these studies and the fact that the palatal surface of teeth, which suffers predominantly, is less susceptible to efficient toothbrushing.

In most studies the examined subjects showed differing resistance to dental erosion, which may be explained by intersubject variation in protective factors such as salivary flow rates and buffer capacity, which will be discussed later.

1.4.3 Abrasion

One of the most important factors aggravating the erosive effect of intrinsic and extrinsic acids on dental enamel is mechanical wear of its superficial layer softened by the acidic challenge. This mechanical wear includes simple mechanical friction by opposing teeth, lips, cheeks and especially the tongue, as well as the consumption of hard texture food products and the effect of conventional oral hygiene procedures such as toothbrushing (Sognnaes, 1963). The former process is known as attrition and the latter as abrasion. In most cases of dental erosion on labial and sometimes on lingual tooth surfaces, the greatest exacerbating influence on toothwear is expected to be delivered by brushing with abrasive toothpaste. The use of abrasive-containing dentifrice causes abrasive effects even on sound enamel, whilst brushing without dentifrices provokes the formation of an organic-mineral protective membrane on the enamel surface (Kuroiwa *et al*, 1993). In a similar study on acid-etched enamel, the nature of the process, which is very similar to dental erosion, showed that brushing without toothpaste induced the remineralisation of softened enamel by depositing salivary components, whilst use of paste led to abrasion of the lesion (Kuroiwa *et al*, 1994).

It is to be expected that the rate of enamel abrasion caused by toothpaste is related to the hardness of the enamel and the abrasive agent (Wright, 1969). This statement could be true with regards to the abrasive agent but the study of

sound mature permanent enamel with different levels of hardness revealed no significant difference in toothwear due to brushing abrasion in the work by Bartlett *et al* (1994). Different relationships may be found if the tooth surface has been attacked by an erosive agent. There is data suggesting that significantly more dental hard tissue is removed during brushing with abrasive toothpaste if this procedure follows the consumption of erosive foods *in vivo* or artificial production of erosive lesions *in vitro* (Schweizer-Hirt *et al*, 1978; Davis and Winter, 1980; Attin *et al*, 1997). This confirms that the hypomineralised and softened surface of eroded enamel is liable to result in accelerated tissue loss, at least to the level where the all damaged enamel layer would be removed (Wright, 1969) or rehardened due to deposition of salivary or remineralising substrates.

Human saliva is hypersaturated with respect to calcium and phosphate, the details of which will be discussed later. This allows enamel hydroxyapatites to restore their density partially or completely. Abrasion resistance of eroded enamel continuously increases with the remineralisation time (Jaeggi and Lussi, 1999; Attin *et al*, 2000) and depends on whether it takes place in the presence of sufficient concentration of fluoride ions. Less wear is produced on eroded enamel during brushing in the presence of the fluoride toothpaste than in the presence of non-fluoride toothpaste (Bartlett *et al*, 1994). When abrasive containing toothpastes are replaced with non-abrasive gels containing adequate amounts of fluoride, the degree of toothwear relating to abrasion reduces to a very low level (Attin *et al*, 1999). This suggests that patients suffering from dental erosion

should use a non-abrasive fluoridated gel if they clean their teeth immediately following an erosive attack.

1.4.4 Factors characterising the protective properties of oral environment

1.4.4.1 Effect of saliva

Saliva plays an important role in oral health maintaining the integrity of the oral tissues especially hard tooth tissues. The protective property of saliva against caries attack is well known. Lagerlöf and Oliveby (1994) reported in their review that the most important anti-caries factors of saliva is its rate of flow and amount of fluoride ions and less important is its buffering ability, calcium and inorganic phosphate content, which mainly depend on salivary flow rate. The major caries processes take place in dental plaque and under the enamel surface, where buffering capacities differ from those in whole saliva. In another review Edgar *et al* (1994) characterised anticaries abilities of stimulated saliva as mostly due to salivary clearance, buffering power and degree of saturation with respect to tooth mineral.

The chemical process of the erosive challenge of acids on tooth enamel is different from that of caries as it takes place on an enamel surface not covered with dental plaque. The influence of saliva on this process could have another mechanism. Some studies *in vitro* and *in situ* have shown that saliva protects against artificially produced dental erosion. Storing tooth samples in fresh human saliva (*in vitro*) or in the human mouth between cyclic applications of the erosive agent significantly reduced the severity of dental erosion compared with deionised water (Hall *et al*, 1999). An *in vivo* study on healthy volunteers after

consuming potentially erosive acid-containing drinks and measuring pH on the surface of the tongue reported that it returned to values above 5.5 in less than two minutes (Meurman *et al*, 1987) suggesting the high clearing and buffering capacities of saliva.

The main protective factors against tooth erosion are probably salivary buffers, mainly due to bicarbonates, salivary flow rate, as in the simple diluting and removing of destructive acids from exposed tooth surface, and the viscosity of saliva. The results in the literature are controversial on these important aspects. In studying patients with different types of dental erosion some authors revealed strong correlations between salivary flow rate, buffering capacity and incidence of this pathological condition. Wöltgens *et al* (1985), on a sample of patients with idiopathic erosion and Järvinen *et al* (1991) on a case-control study, showed that the risk of erosion was high in individuals with a low unstimulated salivary flow rate. The same correlation for flow rates and buffer capacity was reported in the work by Bevenius and L'Estrange (1990), but this was observed on a small sample of subjects suffering from tooth surface loss. Linkosalo and Markkanen (1985) and Gudmundsson *et al* (1995) reported that subjects without erosion had significantly higher buffering capacity for stimulated saliva than those with erosion. However no relationship between the severity of toothwear and salivary flow rate and/or buffering power of stimulated saliva was observed in the epidemiological study on 11-12-year-old schoolchildren (Bartlett *et al,* 1998), on patients with pathological gastroesophageal reflux in stimulated and unstimulated saliva (Meurman *et al,* 1994) and in the work of Milosevic and

Slade (1989) on bulimic/anorexic patients with frequent vomiting in unstimulated saliva. In dental erosion an important factor is probably unstimulated saliva because it flows for the most of the day (Sreebny, 1990) and the bulk of possible remineralisation of softened element by erosive agent enamel is expected to occur during conditions, when resting salivary conditions predominate (Jaeggi and Lussi, 1999).

In a comparison of two groups of patients suffering from bulimia nervosa with and without noticeable toothwear Milosevic and Dawson (1996) did not find a difference in stimulated salivary flow rates and bicarbonate concentrations, but the viscosity of saliva was significantly greater in bulimic subjects with toothwear. The same relation between viscosity of saliva and erosion was described by Mannerberg in 1963. However Meurman *et al* (1994) reported no difference in the range of viscosity between compared groups. Another interesting finding in 1949 reported a positive statistical correlation between the severity of human erosion and the salivary citrate content (Zipkin and McClure).

1.4.4.2 Effect of salivary pellicle

There is also evidence of the protective effect of salivary pellicle on underlining enamel erosion *in vitro* (Mannerberg, 1962; Nieuw Amerongen *et al*, 1987; Meurman and Frank, 1991) and *in situ* (Amaechi *et al*, 1999b). The latter

study showed that the thickness of acquired pellicle is site-specific in the dental arch and provides one possible explanation for the selective location of dental erosion in the human mouth. There was also an observed strong negative correlation between the degree of erosion and pellicle thickness.

All findings mentioned above, regarding oral environmental factors, show the sophisticated miscellaneous nature of dental erosion and the necessity to continue research in this field.

1.5 Methods and techniques for studying dental erosion

1.5.1 Overview

As clinical trials on humans to study dental erosion are limited by difficulties in the accurate quantification of intra-oral wear and by ethical reasons, most of the studies on this subject have been performed *in vitro* and on laboratory animals *in vivo*. Several techniques have been developed to produce and measure hard dental tissue loss both *in vivo* and *in vitro*. In his review, Grenby (1996a) described those procedures, which were used in production and quantification of toothwear since dental erosion became a focus of research in laboratories worldwide. Some new techniques to assess dental erosion, which were published recently, are presented below and in some cases could be employed *in vitro* as well as *in vivo*.

1.5.2 *In vivo* methods

A clinical index for evaluating and monitoring the progression of erosion has been developed (Larsen *et al*, 2000), which uses the casts of the teeth and demonstrates precise surface reproduction. A six-grade scoring system is used to assess each tooth surface and includes the presence of Class V restorations,

which all together represent the severity of tooth loss and could be suitable for data analysis.

A reproducible *in vivo* method to assess toothwear was proposed by Bartlett *et al* (1997) using contact laser profilometry, which measures the change of depth around a reference metal disc fixed to the palatal surface of upper incisors. A non-destructive technique, which possibly could be used *in vivo*, was published by Huysmans and Thijssen (2000). They suggested measuring the thickness of enamel by use of an ultrasonic pulse-echo instrument, although this method was not sufficiently accurate to quantify small changes in enamel tissue loss during the initial stages of dental erosion that could be detected by other techniques.

1.5.3 *In vitro* methods

Transverse microradiography (TMR) was demonstrated by de Josselin de Jong *et al* (1987) to measure mineral loss in caries research and was first used in the assessment of dental erosion by Hall *et al* (1997a) and developed by Amaechi *et al* (1998a). This technique allows for the measurement of very precise degrees of mineral loss as well as the depth of the crater of acid erosion and depth of subsurface demineralisation, which has an application for use *in situ* studies. This technique is widely recognised as the "gold standard" in dental research, for

quantifying changes in mineral in dental hard tissues, however, it is time consuming and because of the need for sample preparation can be considered to be a destructive procedure.

The atomic force microscope has also been utilised (Parker *et al*, 1998; Finke *et al,* 2000) to investigate initial qualitative and quantitative changes in surface morphology of teeth exposed to acidic soft drinks and was shown to be a suitable tool for measuring the early stages of enamel demineralisation.

Millward *et al* (1995) introduced a method, which used a replica impression technique followed by non-destructive scanning electron microscopic examination. The *in vitro* experimental model for erosive lesion formation imitated the *in vivo* situation during an acid attack. Specially designed devices provided the possibility of studying samples challenged with erosive agents under controlled conditions: temperature regime, exposure time, acid clearance by salivary washing, cycle contact of corrosive and remineralising factors. Amaechi *et al* (1999a) demonstrated a simple *in vitro* model for dental erosion including oral conditions that exist on the tooth surface *in vivo*. At regular intervals six times per day for 24 days tooth samples were immersed in orange juice for 5 minute periods (total exposure 12 hours) and for the remainder of the day were stored in a solution of artificial saliva thus simulating the 6-times a day consumption of orange juice.

1.5.4 Optical visualisation methods

Newer methods introduced for visualisation and measurement of enamel demineralisation in caries studies, such as confocal microscopy (Fontana *et al*, 1996), quantitative light-induced fluorescence (de Josselin de Jong *et al*, 1995) and optical coherence tomography (Colston *et al*, 1998) have been suggested as potential tools for the study of dental erosion.

Quantitative light-induced fluorescence (QLF), a non-destructive technique for the longitudinal evaluation of early caries demineralisation uses the effect of natural fluorescence of the tooth hard tissues to calculate the difference between sound and demineralised enamel. Pretty *et al* (2004) showed that QLF was able to detect and longitudinally monitor acid erosion *in vitro*. They reported a strong positive correlation in the measurement of mineral loss using both QLF and transverse microradiography. This suggests that fluorescence loss of erosive lesions could be of potential importance in the application of this non-destructive method in dental research.

Confocal laser scanning microscopy is a non-destructive, 3-dimentional microscopic tomography technique and has been used in the work by Duschner *et al* (2000) to visualise early changes in the enamel surface after an erosive attack with a carbonated cola drink.

Optical coherence tomography (OCT) is a new imaging technique that uses low coherence interferometry to selectively eliminate the multiple scattering component of backscattered light, making it possible to acquire two dimensional images of dental enamel at high resolution. Amaechi *et al* (2001) using the A-scan of OCT technique, obtained by optical pathlength spectroscopy, to measure the depth resolved reflectivity of the tooth tissue both in sound enamel and caries like lesions. They showed that OCT could quantitatively monitor the mineral changes in a caries lesion longitudinally.

1.5.5 *In situ* models

As mentioned previously, studies on dental erosion in humans are not widely carried out because of problems in the accuracy of measurement of tissue loss/gain and for ethical reasons. Probably the best method to make demineralisation/ remineralisation studies of dental erosion closer to the natural intra-oral environment could be the use of *in situ* models. This technique was introduced by Koulourides and Volker in 1964 and since that time, it has become widely used and modified for use in caries research and more recently in experiments on dental erosion.

In situ modelling involves the use of enamel slabs cut from animal or human teeth mounted in specially designed removable or fixed appliances worn

in the human mouth. The first citation of the use of an intra-oral device bearing tooth slabs softening enamel with acid drink and subsequent rehardening was in the work by Gedalia *et al* (1991). Ten subjects wore removable orthodontic devices with mounted slabs and rinsed a cola drink around their mouths for one hour followed by five minute chewing of a hard cheese or paraffin wax. Cheese consumption showed a significantly greater remineralisation effect (quantified using microhardness and Scanning electron microscopy) than just the stimulation of saliva alone.

The demineralisation potential of orange juice (West *et al*, 1998) and low erosive blackcurrant juice drink (Hughes *et al*, 1999b) was studied with prechosen precautions in comparison to previous work: if a safety margin of loss of 20 μm of enamel was achieved during the intra-oral procedure, subjects were excluded from the trials. Rugg-Gunn *et al*, (1998) designed *in situ* appliances in a way that was possible to insert the two enamel slabs mounted on it into two different solutions outside the mouth, thus imitating consumption of erosive drink without any damage to the natural teeth of volunteers. In another study (Jaeggi and Lussi, 1999) dental erosion was previously produced on slabs *in vitro* and then remineralisation capacities of human saliva were tested *in situ*.

Most existing removable intra-oral systems have several problems. Tooth slabs are often not placed in sites naturally susceptible to dental erosion, plus they could be subjected to abrasion effects from the surrounding oral tissues, which are not always common in these particular sites *in vivo*, they could be

removed easily and readily by subjects due to discomfort and lack of supervision. All these and other possible subjective and objective factors can lead to the non-compliance of the study protocol and affect final results (Amaechi *et al*, 2000). To avoid such problems as well as to decrease the cost of the model a simple *in situ* system was developed. Enamel slabs, covered at the edges with composite to protect the surface to be investigated against abrasive forces, were cemented to buccal or lingual surface of teeth (Amaechi *et al*, 2000). The results of this new fixed intra-oral appliance trial showed that volunteers got accustomed to the model very quickly; they were able to maintain satisfactory oral hygiene throughout the experimental period. Data showing enamel remineralisation evaluated using TMR were obtained and it is anticipated that this model will prove to be useful for the assessment of the factors influencing the remineralisation processes taking part in eroded enamel.

1.6 Prevention and remineralisation of dental erosion

1.6.1 Alteration to the composition of eroded agents

Numerous studies have been conducted in the field of dental caries to look at aspects such as decreasing the demineralisation potential of foodstuff (Shrestha *et al*, 1982; Rankine *et al*, 1989), improvement of the protecting properties of dental enamel (ten Cate and Duijsters, 1982; Arends and Christoffersen, 1986), restoration of the early-developed lesions (Koulourides *et al*, 1965; Collys *et al*, 1993). All these factors, which will be discussed, may be of importance in erosion research. Both caries and erosion share a common underlying principle of acid attack on enamel hydroxyapatites. The major difference, however, being that caries develops in the micro-space under the surface of dental plaque in the presence of bacteria.

Certain steps to modify the formula of acidic drinks causing dental erosion have been undertaken since the relationship between them has been found. Adding fluoride in different concentrations into some beverages, which were used to produce erosion *in vivo* on laboratory animals and *in vitro*, demonstrated a high protective characteristic of this ion against the erosive challenge (Restarski *et al*, 1945a; Spencer and Ellis, 1950; Holloway *et al*, 1958; Gedalia *et al*, 1981; Sorvari *et al*, 1988). Amaechi *et al* (1998b) reported the additive effect of fluoride and xylitol on the inhibition of erosion. However,

decrease of mineral loss with fluoride only and xylitol only was not significant in their *in vitro* study.

The effect of calcium and phosphate concentrations in fruit juices and carbonated beverages on enamel demineralisation encouraged some investigators that a similar approach could be used to decrease the erosive potential of these drinks and other acid-containing foodstuff. Saturating experimental solutions with calcium and/or phosphate has been observed to significantly decrease the degree of dental erosion, when compared with unsaturated drinks. Wagg *et al* (1965) reported the effect of adding phosphate, calcium lactate and calcium carbonate to water ice (lolly) formulations, Beiraghi *et al*, (1989) with calcium lactate on Coca-Cola, Reussner *et al* (1975) and Larsen and Nyvad (1999) with monocalcium phosphate on fruit and carbonated beverages. Hughes *et al* (1999b) reported the low-erosive properties of a newly developed low pH blackcurrant juice drink containing calcium carbonate. Calcium lactate became the more popular additive in low-caries and low-erosive drinks and food, as it is non-toxic and tasteless as well as quite soluble (Beiraghi *et al*, 1989; Rankine *et al*, 1989; Kashket and Yaskell, 1997).

1.6.2 Strengthening the dental enamel surface prior to erosive attack

It is well known from caries studies that fluoride makes enamel more resistant to acid after topical application (Ostrom *et al*, 1984; Eronat *et al*, 1993). The effectiveness of fluoride toothpaste (Davis and Winter, 1977), fluoride varnish and sodium fluoride solution (Sorvari *et al*, 1994) against initial enamel erosion has been shown. According to these studies the topical application of fluoride cannot totally prevent erosion, but using fluoride prior to exposure to the erosive agent may significantly increase the hardness of dental enamel and thus inhibit the enamel surface softening during the early stages of dental erosion.

1.6.3 Remineralisation of eroded lesion

Another area, which is of great importance in controlling the process of hard dental tissue loss, is the possibility of restoring the structure and the volume of damaged enamel hydroxyapatites. The ability of enamel to gain minerals and partly or totally remineralise after an acid attack was demonstrated by Albert and Grenoble (1971) *in vivo* and *in vitro* by Amaechi *et al* (1998a). As previously discussed, the hardening of the demineralised tooth surface depends on several factors: quality of remineralising environment – human saliva/artificial saliva,

application of fluoride containing supplements, presence or absence of abrasive forces.

As previously shown, saliva has the ability to protect against erosive challenges, by increased flow rates, buffering capacities, viscosity, amount of calcium, phosphate and fluoride. When one or more of these factors varies the subject can become more or less susceptible to an erosive attack. Low salivary flow rate and the lack of its quality displayed in low buffering power and/or high viscosity might be noticed in patients suffering from xerostomia, induced for example by radiation, some therapeutic drugs and diseases such as Sjögren's syndrome. Besides their susceptibility to dental caries (s'-Gravenmade *et al*, 1981; Baudet-Pommel *et al*, 1994) they can have a high risk of developing dental erosion possibly due to increased consumption of acidic fluids, such as fruit juices in order to stimulate salivary flow and obtain symptomatic relief. In such cases stimulation of natural saliva by chewing gum (Davies, 2000) and/or using a saliva substitute may be advantageous.

The ability of artificial saliva to mimic the natural human oral fluid and have remineralisation potential on early lesions was first described by Shannon *et al* (1977, 1978). Sufficient concentration of calcium, phosphate and fluoride (Shannon and Edmonds, 1978) provide softened enamel with minerals, whilst the presence of mucin or the more efficient carboxymethylcellulose (Gelhard *et al*, 1983) is necessary for maintaining the viscosity and lubricating properties of saliva substitute and its retention in oral cavity. It also has to have ionic strength

equivalent to natural saliva (Gelhard *et al*, 1983), pH above the critical value for hydroxyapatite dissolution (Kidd and Joyston-Bechal, 1984) and an adequate buffering capacity. Most publications in this area relate to dental caries and there is a little data available on the effect of saliva substitutes describing their remineralisation influence on dental erosion *in vivo* or *in situ.* However, the effectiveness of artificial saliva to induce remineralisation of artificially softened enamel *in vitro* has been reported (Amaechi *et al*, 1998a, 1999a).

Apart from using saliva analogues for strengthening eroded enamel it could be useful to take advantage of other remineralising remedies, which exhibit an effectiveness against caries. The most popular among them are fluoride-containing toothpastes and gels, rinsing solutions, dental varnishes and chewing gums. Treatment with fluoride toothpaste significantly increased the hardness of an acidic drink-etched enamel in a laboratory study (Munoz *et al*, 1999), but precautions should be taken as abrasive forces of the toothbrush, when applied, can lead to surface wear of softened enamel even in presence of fluoride ions. This problem could be minimised by using nonabrasive or low abrasive toothgels containing fluoride. The strengthening effect of other fluoride-containing vehicles on eroded enamel is yet to be investigated.

1.7 Summary

- There is no doubt that the problem of dental erosion is perhaps the most important factor in toothwear and has increased over the past few decades. This may be the result of increasing consumption of acid containing drinks as well as increased awareness of the condition.

- Epidemiological data on dental erosion, such as prevalence and severity in different age and factor-related groups, typical location on the dental arch, vary between different surveys probably because a range of different diagnostic indices and examination techniques have been used and in addition early identification of this pathological condition may be problematic.

- Two major factors could be contributing to the development of erosion: dietary and gastric acids, the role of which on dental health is well documented.

- Certain changes in saliva contribute to the loss of the enamel surface in dental erosion: flow rate, buffering capacities, concentration of calcium, phosphate and fluoride. Further research is required if discrepancies are to be resolved.

- Transverse microradiography is a useful technique to measure loss/gain of dental mineral tissues and its use will contribute to the development of *in vitro* and *in situ* models to study dental erosion.

- The most important areas in research for prevention of dental erosion in addition to dental education are the reduction of the erosive potential of acidic beverages and developing effective methods and substances, which help to increase acid resistance of sound enamel and quick remineralisation of enamel softened by acid attack.

- As there are many intrinsic and extrinsic factors, which contribute to dental erosion, the nature of this condition is complicated and difficult to study.

1.8 Aims of this thesis

This thesis aims to develop *in vitro* model systems, which may be used to investigate dental erosion, and to use these model systems in the evaluation of QLF as a tool for quantifying early dental erosion.

1.9 Objectives

1. Optimising the remineralising capabilities of remineralising solution in eroded dental lesions through variations in calcium lactate and fluoride;

2. Evaluating QLF as a diagnostic tool to be used in the quantification of early dental erosion;

3. Evaluating the erosive potential of a commercially available calcium containing orange drink *in vitro*.

Chapter 2

REMINERALISATION POTENTIAL OF SOLUTIONS CONTAINING VARYING CONCENTRATIONS OF CALCIUM ON ERODED LESIONS

UNIVERSITY OF
LIVERPOOL

2.1 Introduction

It is well established from caries studies as well as from erosion data that the presence of calcium, phosphate and fluoride ions can significantly increase the remineralisation process in early enamel lesions and in some cases even reverses them (Koulourides *et al*, 1965; Shannon and Edmonds, 1978; Collys *et al*, 1993; Amaechi *et al*, 1998a). These nonorganic substances are the main active components, when considering the protective and remineralising potential of natural saliva (Robinson *et al*, 2000), in addition a range of artificial remedies have shown promise both *in vitro* and *in vivo*, and some of these are already available on the market (Addy and Moran, 1997; Marinho *et al*, 2003). Caries studies are still the most widely investigated topic in dental research and there has been much interest in the field of mass caries prevention (Marthaler, 2004) and the mechanisms involved in the caries process. In common with dental caries, dental erosion principally involves changes in mineralised dental tissues and therefore researchers have applied knowledge, skills, and technology used in the study of dental caries to the process of dental erosion (Deery *et al*, 2000). The problem of dental erosion is nowadays in focus due to a number of case reports and prevalence studies suggesting that the significant role in the aetiology of this disease is dietary acids (Eccles and Jenkins, 1974; Lussi *et al*, 1991; Nunn, 1996). The second major causal factor of tooth erosion is the damaging effect of the low pH gastric acids, which frequently appear in the oral cavity of patients

suffering with gastrointestinal problems and psychological conditions such as anorexia nervosa and bulimia nervosa (Hellström, 1977; Gilmour and Beckett, 1993; Barlett *et al*, 1996).

There has been considerable interest in the extrinsic factors, which cause erosion and in particular the formulation of drinks has been a major focus of this interest. Data are available, which suggest that changing the composition of drinks e.g. varying ionic content, increasing pH and altering the buffering capacity of beverages has the ability to reduce the erosive potential and thus minimise the demineralising effect on human enamel (Wagg *et al,* 1965; Beiraghi *et al*, 1989; Reussner *et al,* 1975; Hughes *et al,* 1999a). Clearly it is virtually impossible to employ a similar strategy with gastric acids. One possible preventive approach is to reduce the corrosive outcome of gastric acids on enamel and dentine by hardening these tissues prior to an acid attack or to quickly remineralise the softened surface immediately after the acid attack. For these purposes, dentifrices, mouthrinses or saliva substitutes containing certain active ingredients may be advantageous. However, the use of abrasive-containing dentifrice may abrade sound enamel and when teeth, which have been softened by an acid attack, are brushed, significantly greater removal of tooth tissue is observed (Kuroiwa *et al*, 1994; Attin *et al*, 1997). There are several possible ways to avoid excessive toothwear if, for example, a patient prefers to brush their teeth after a vomiting episode. One approach would be to ensure low-abrasive toothpastes/gels are used, which could give rise to a lower degree of enamel loss than with conventional toothpastes. In addition, it was also demonstrated that

adequate amounts of fluoride within the dentifrice (preferably acidified gel) was also of importance (Attin *et al*, 1999). The other approach relies on the remineralising properties of human saliva, which is hypersaturated with respect to calcium and phosphate (Larsen and Pearce, 2003). Abrasion resistance of eroded enamel *in vivo* continuously increases with the remineralisation time when a sufficient gap in time occurs between erosive attacks and following toothbrushing that allows partial or complete restoration of the density of enamel hydroxyapatites (Jaeggi and Lussi, 1999; Attin *et al*, 2000; Eisenburger *et al*, 2001). The degree and speed of surface recovery depends on whether the remineralisation process takes place in the presence of a sufficient concentration of fluoride (Lagerweij and ten Cate, 2002; Hughes *et al*, 2004). Whilst the concentration of fluoride in human saliva is relatively low (0.01 - 0.05 ppm), and the exposure of the teeth to acid attack may be prolonged (especially in those with gastric reflux), the rapid removal of acid residuals is very important. The use of specially formulated mouthrinses, designed to promote remineralisation in these patients, may be advantageous. In order to optimise their remineralising properties the composition of these mouthrinses should include fluoride, calcium and phosphate ions, which play a major role in enamel remineralisation (Larsen and Jensen, 1994). It is very important to determine the most effective concentration of active ingredients in these artificial salivas as relatively low amounts of calcium and fluoride could increase its remineralising potential (Collys *et al*, 1993; Larsen, 2001; Hicks *et al*, 2004). On the other hand, excessive concentration of these ions could lead to rehardening of only the

superficially softened layer of the lesion thus blocking access to the subsurface demineralisation and leaving it hypomineralised (Arends *et al*, 1992).

Calcium in the form of lactate salts has been reported to be an effective anticaries agent and has been used successfully as an additive in low-caries and low-erosive drinks and food. In this form it is non-toxic and tasteless as well as being quite soluble (van der Hoeven *et al*, 1989; Beiraghi *et al*, 1989; Kashket and Yaskell, 1997).

2.2 Aim and Objectives

2.2.1 Aim

The aim of the study described in this chapter was to assess the effects of varying calcium concentration in a remineralising solution on artificially produced dental erosion *in vitro*.

2.2.2 Objectives

The objectives of this study were to:

- investigate the remineralising effect of calcium lactate on artificially produced erosive lesions *in vitro*;
- establish the optimum concentration of calcium ions in remineralising solution;
- compare the remineralising effect of newly formulated solutions with natural saliva on eroded lesions.

2.3 Materials and methods

2.3.1 Tooth selection and preparation

Freshly extracted human molars, free from caries, enamel malformations and visible cracks, were collected. Thirty five teeth were selected for the study; they were scrubbed to remove debris and soft tissue and polished with pumice and with a 1,200-grit abrasive sandpaper (Wet or Dry Sandpaper, 151 Products Limited, Manchester, UK) to remove remaining fine organic contaminants. Each tooth was then coated with two layers of acid-resistant nail varnish (MaxFactor®, Procter and Gamble, Weybridge, UK) except for a rectangular window (8x2 mm) in the middle of the buccal surface of the tooth Figure 2.1.

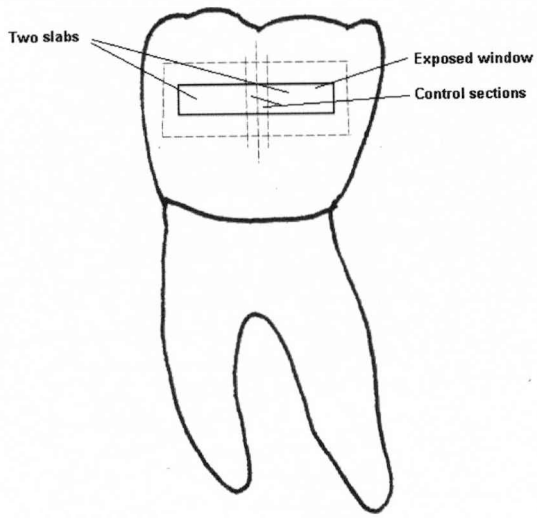


Figure 2.1 Schematic illustration of exposed window

2.3.2 Creation of erosive lesions

Artificial eroded lesions were produced by attaching the tooth samples described above to glass rods (Figure 2.2) using thermoplastic impression material (Green Stick Impression Compound, Kerr, Sybron, USA) and then immersing them at room temperature in orange juice, purchased from a supermarket (Tesco Value, Tesco, UK; batch no X1075), for 90 minutes. The orange juice was continuously stirred using a MR 3000 magnetic stirrer (Heidolph, Germany) at a speed of 200 rpm. The pH of orange juice was tested at

room temperature with a combination pH electrode (Orion, Boston, MA, USA) connected to an Alpha 500 pH meter (Aqua Scientific, Kent, UK).

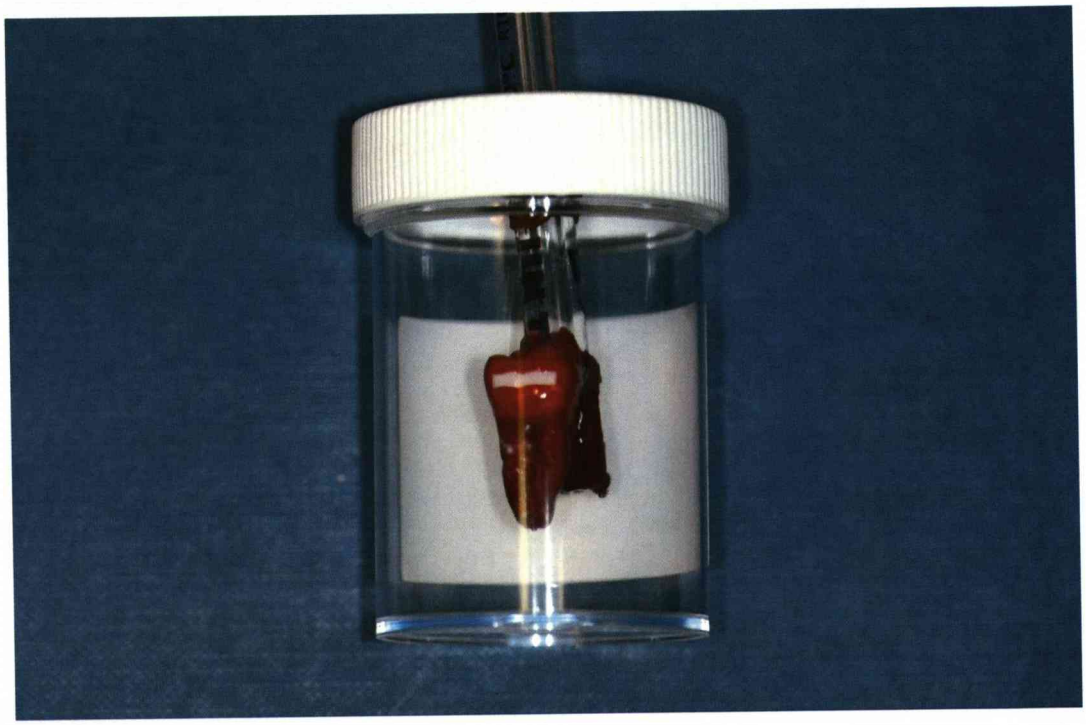


Figure 2.2 Tooth prepared for *in vitro* erosion

2.3.3 Preparation of solutions

The remineralising solutions were prepared using a modified formula of artificial saliva based on that described by Amaechi *et al* (1998a). This contained sodium carboxymethyl cellulose, 0.4 g/L; KCl, 0.625 g/L; NaF, 0.113 mg/L (0.05 ppm of F^-); $MgCl_2 \cdot H_2O$, 0.03 g/L; K_2HPO_4, 0.102 mg/L; KH_2PO_4, 0.041 mg/L; methyl-p-hydroxybenzoate, 2.0 g/L. The solution was modified by the addition of calcium lactate (instead of calcium chloride used in the original formulation). The amount of sodium carboxymethyl cellulose was reduced from that used in the original composition in order to produce a solution with a viscosity more appropriate for use as an intra-oral rinse. The stock solution was used to prepare six solutions differing only in calcium concentration. The first group had no added calcium and served as a negative control. The remaining solutions had calcium lactate added to give 50 mg/L calcium ions in the 2nd group, 100 mg/L in 3rd, 250 mg/L in 4th, 500 mg/l in 5th and 1000 mg/L in 6th group. The pH was adjusted to 6.7 using KOH. Fresh natural human saliva was used as a positive control. The natural saliva collection procedure is described below.

2.3.4 Preparation of samples

Slabs, comprising eroded lesions and surrounding sound enamel, were cut from each molar using a water-cooled diamond-wafering blade (Buehler, UK). Each slab was cut in half and control sections were cut from each slab using a water-cooled diamond wire saw (Well, Walter Ebner, Switzerland) Figure 2.3. These were retained for microradiography. The remaining slabs were randomly allocated into 7 groups with 9 slabs in each.

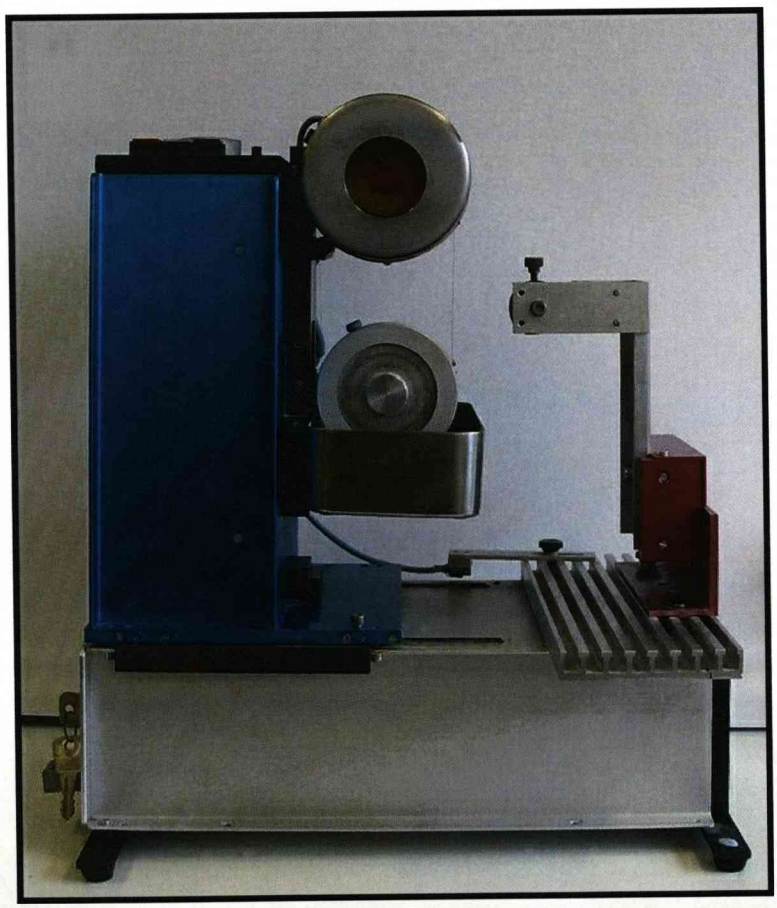


Figure 2.3 Wire saw machine

All slabs obtained in this way were mounted on glass rod with acid-resistant nail varnish (MaxFactor®, Procter and Gamble, Weybridge, UK) and secured in 30 ml universal tubes, Figure 2.4.

Figure 2.4 Enamel slabs mounted on a glass rod in preparation for the remineralisation cycle

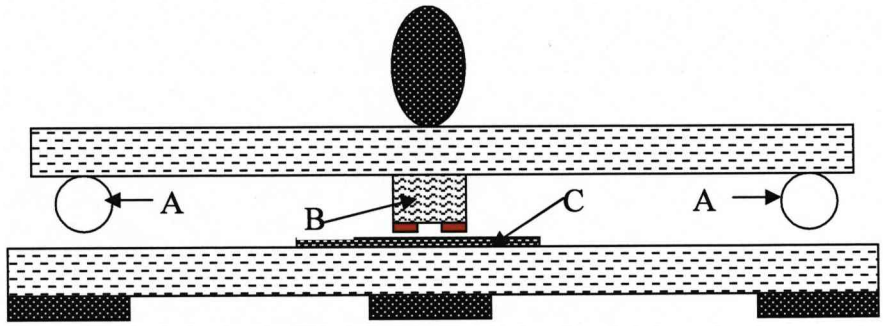
2.3.5 Experimental procedure

The specimens were exposed to test solutions of artificial and natural saliva on a SB1 blood tube rotator (Stuart Scientific, UK) at a speed of 30 rpm in an UM300 oven incubator (Memmert, Schwabach, Germany) at 37°C, Figure 2.5. Fresh stimulated natural saliva was collected mid-morning each day from a single subject by chewing nonflavoured gum base (Espoo, Finland). Immediately following collection, natural saliva was centrifuged at 12,000 rpm for 30 minutes at -4.0°C (Model J2-21 centrifuge, Beckman, UK). All solutions were changed daily. The experiment was carried out for 28 days.

Figure 2.5 Enamel samples in remineralising solution on tube rotator in oven incubator

2.3.6 Lesion sectioning and grinding

Using a water-cooled diamond wire saw (Well, Walter Ebner, Switzerland) the samples were cut into thin sections bearing the eroded area and adjacent sound enamel. The sections obtained in this way were examined using transverse microradiography (TMR). All sections were mounted on brass anvils with nail varnish and polished from both sides with a diamond disk (Figures 2.6 and 2.7) to give plano-parallel specimens of 100 μm thickness.



A - Ball bearings

B - Brass anvil with tooth sections fixed with nail varnish

C - Polishing diamond disc

Figure 2.6 Schematic illustration of equipment used for producing plano-parallel tooth sections

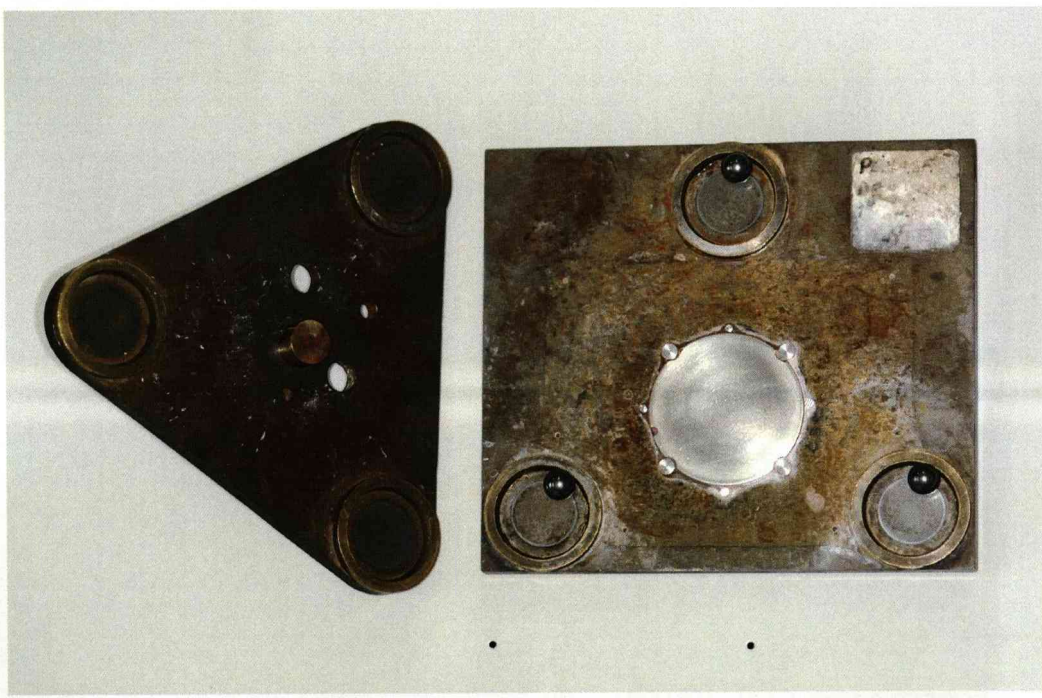


Figure 2.7 Photograph of equipment shown in Figure 2.6

2.3.7 Transverse microradiography

Following cycling in remineralising solutions, sections were cut from each slab and microradiographed. Mineral loss (ΔZ) and lesion depth (ld) were quantified using TMR (Figure 2.9) and the data analysed by paired t-test, one-way ANOVA and multiple comparison ($\alpha = 0.05$). The percentage change in mineral content (ΔZ) and lesion depth (ld) of each lesion was calculated as follows:

$$\%\,\Delta Z = \frac{\Delta Z\,(\text{control}) - \Delta Z\,(\text{test})}{\Delta Z\,(\text{control})} \times 100\%$$

$$\%\,\text{ld} = \frac{\text{ld}\,(\text{control}) - \text{ld}\,(\text{test})}{\text{ld}\,(\text{control})} \times 100\%$$

The sections were mounted on a microradiographic plate-holder bearing an aluminium stepwedge (25 μm steps), Figure 2.8. The microradiographs were taken with a 15-min exposure on Kodak high-resolutions plates (type 1A) using a Cu (Kα X-ray source operating at 25 kV and 10 mA at a focus-specimen distance of 30 cm. The plates were developed using standard techniques. The microradiographs were subjected to image analysis under a Leica DMRB microscope (Leica, Germany). The image was captured at a magnification of 20x/0.40 via a CCD video camera (Sony, Japan) connected to a computer (Viglen PC, UK).

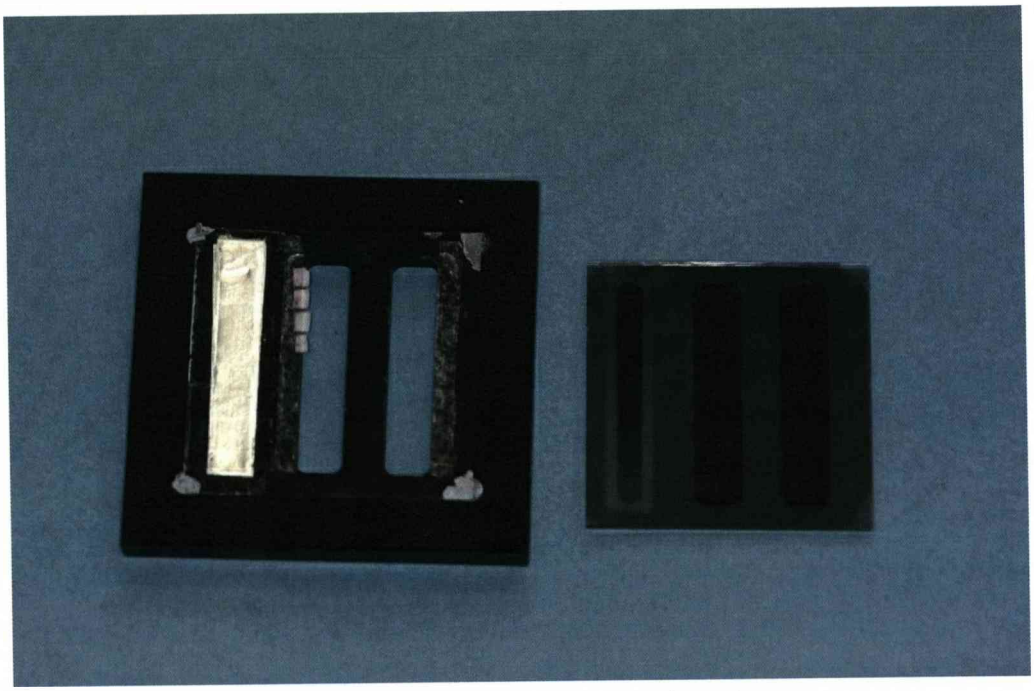


Figure 2.8 Microradiographic plate-holder together with developed microradiographic plate

The integrated mineral loss (vol%·μm), lesion depth (μm) and the depth of crater in control and final erosions were assessed by a two-step image analysis technique (Amaechi *et al*, 1998a) by means of a software package (TMRW v. 1.22, Inspektor Research Systems BV, Amsterdam, The Netherlands), Figure 2.9.

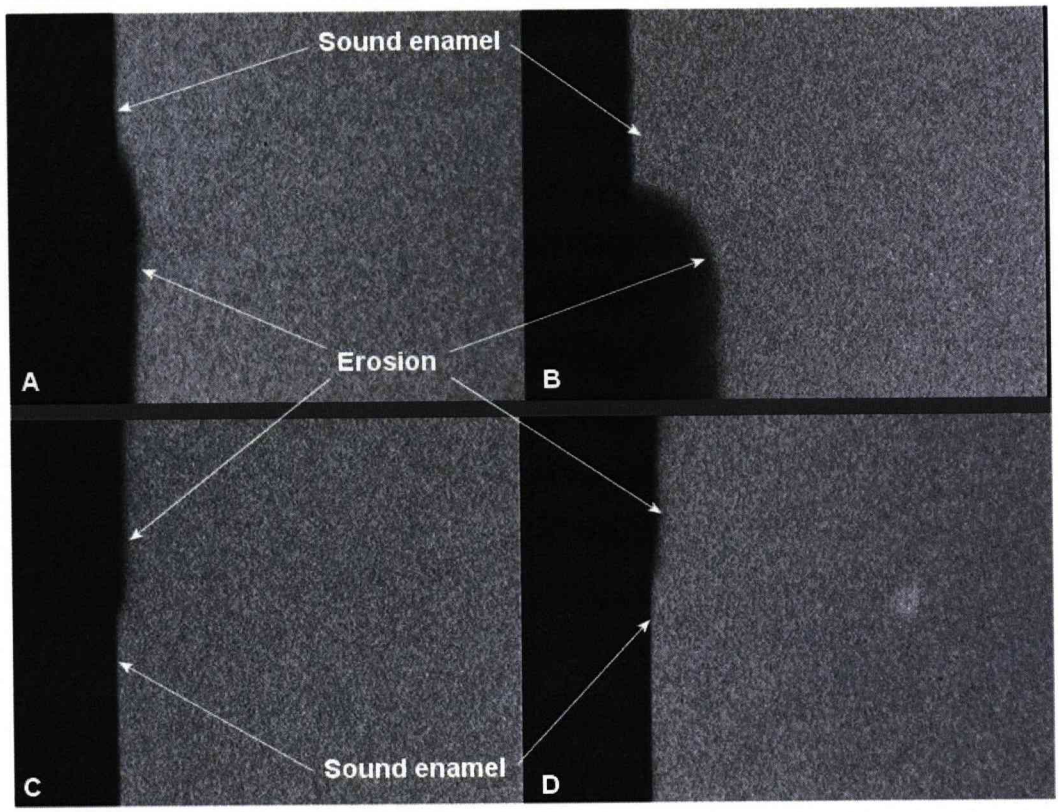


Figure 2.9 TMR images showing progression of enamel erosion

2.4 Results

The mean pH of orange juice used to produce the initial eroded lesions was measured to be 3.65 ± 0.01.

Table 2.1 shows the mineral loss (vol%·μm) and Table 2.2 shows the lesion depth (μm) of the nine control specimens allocated to the experimental groups. The mean mineral loss ranged from 580.2 ± 194.8 in the 1000 ppm Ca group to 699.2 ± 168.0 in the 100 ppm Ca group. Neither mineral loss nor lesion depth in the seven experimental groups differed significantly ($p > 0.05$).

Four weeks of exposure to the experimental solutions gave rise to changes in the mineral loss and lesion depth of the specimens, details of which are shown in Table 2.3 and 2.4. The largest observed change in mineral loss was found in 0-ppm Ca group, where an increase in mineral loss to 3214.5 ± 544.4 vol%·μm (Table 2.3) and an increase in lesion depth to 54.8 ± 8.3 μm were recorded. The only significant differences ($p < 0.0001$) were found between the 0-ppm Calcium group and the experimental groups (50, 100, 250, 1000-ppm Ca and natural saliva). There was no significant difference ($p > 0.05$) between any of the experimental groups. A similar pattern was also observed when lesion depths were analysed using the Tukey multiple comparison index with the 0-ppm Ca group giving statistically significant greater ($p < 0.001$) lesion depths that the experimental groups.

Table 2.5 shows the percentage difference in mineral loss as measured by TMR for the experimental groups. In most samples mineral gains were observed (with positive values indicating remineralisation). In the 0-ppm Ca group and in four other specimens mineral losses were observed (indicated by negative values). These were high in the 0-ppm Ca group with a mean of -432.8 ± 204.6 % recorded, which shows demineralisation, which was found to be significantly different ($p < 0.0001$) from that observed in all other experimental groups, when the Tukey multiple comparison index was used.

Similarly the percentage difference in lesion depth shown in Table 2.6 was also found to be significantly different ($p < 0.001$) in the 0-ppm Ca group, when compared with all other experimental groups. No significant differences were found, when calcium was present at any of the concentrations used ($p > 0.05$).

Table 2.1 Control mineral loss, vol%·μm as measured by TMR

Tooth number	Group						
	Calcium concentration (ppm)						Natural saliva
	0	50	100	250	500	1000	
1	686.6	513.0	595.4	703.0	545.6	703.0	934.2
2	684.4	589.2	767.4	674.9	783.7	596.7	758.5
3	894.1	505.7	769.0	754.2	746.0	626.6	577.7
4	538.3	739.6	760.6	359.3	651.4	977.0	977.0
5	758.5	673.3	593.8	747.3	316.5	387.2	300.9
6	583.5	485.7	404.2	550.9	886.8	389.6	554.2
7	621.5	707.6	621.5	514.2	670.5	359.3	920.6
8	359.3	655.6	780.2	698.1	698.1	645.4	465.2
9	710.7	503.7	1001.0	876.4	678.4	536.8	740.0
Mean	**648.5**	**597.0**	**699.2**	**653.1**	**664.1**	**580.2**	**692.0**
SD	**149.9**	**99.0**	**168.0**	**154.1**	**160.9**	**194.8**	**233.3**

Unless marked, differences were not significant between groups at the 5% level.

Table 2.2 Control lesion depth, μm as measured by TMR

Tooth number	Group						
	Calcium concentration (ppm)						Natural saliva
	0	50	100	250	500	1000	
1	14.9	10.9	11.9	14.9	13.1	14.9	23.8
2	36.5	18.8	16.5	15.9	17.2	13.3	13.8
3	20.8	11.5	15.9	14.5	15.0	11.5	12.7
4	11.7	17.0	16.6	8.9	13.7	19.8	19.8
5	13.8	12.7	13.2	15.5	11.7	8.0	8.2
6	11.7	12.1	14.3	14.9	18.8	14.1	14.7
7	14.1	16.4	14.1	10.4	15.1	8.9	22.0
8	8.9	11.6	16.3	15.9	15.9	13.6	9.7
9	14.5	11.6	21.6	18.6	17.0	14.3	16.8
Mean	**16.3**	**13.6**	**15.6**	**14.4**	**15.3**	**13.2**	**15.7**
SD	**8.2**	**2.9**	**2.8**	**3.0**	**2.2**	**3.5**	**5.4**

Unless marked, differences were not significant between groups at the 5% level.

Table 2.3 Final mineral loss, vol%·μm as measured by TMR

Tooth number	Group						
	Calcium concentration (ppm)						Natural saliva
	0	50	100	250	500	1000	
1	2679.1	430.4	464.3	695.5	395.7	525.5	548.7
2	2308.6	582.7	751.9	402.0	634.5	546.3	627.5
3	3663.3	449.4	723.1	588.3	528.8	567.5	560.3
4	3835.3	620.8	410.1	342.7	566.8	821.0	747.1
5	2729.8	528.3	596.1	527.8	302.4	270.7	333.3
6	3724.1	495.4	383.2	476.3	731.7	328.5	492.1
7	3541.6	647.5	504.7	513.8	675.2	312.6	856.9
8	3418.2	620.6	757.6	546.7	562.4	523.6	321.0
9	3030.5	484.0	919.4	672.5	559.8	490.5	431.7
Mean	**3214.5***	**539.9**	**612.3**	**529.5**	**550.8**	**487.4**	**546.5**
SD	**544.4**	**80.5**	**185.2**	**115.1**	**133.2**	**168.3**	**178.8**

*** - Significant difference between 0-ppm Ca and all other groups, $p < 0.0001$ (Tukey multiple comparison index), between other groups differences were not significant at the 5% level.**

Table 2.4 Final lesion depth, μm as measured by TMR

Tooth number	Group						
	Calcium concentration (ppm)						Natural saliva
	0	50	100	250	500	1000	
1	50.9	11.4	11.6	12.2	12.3	12.7	20.8
2	45.1	15.6	15.7	13.3	16.9	15.3	16.8
3	61.7	11.1	13.8	16.8	12.2	12.8	13.2
4	68.9	13.7	8.6	11.3	17.4	19.2	17.3
5	43.5	12.6	12.0	16.4	11.1	11.3	9.7
6	61.0	13.5	10.1	14.5	16.5	10.1	13.7
7	54.3	13.8	14.1	12.6	17.5	10.4	19.4
8	57.2	15.5	18.4	15.2	14.5	13.5	11.9
9	50.3	12.2	17.1	18.0	17.2	17.3	13.3
Mean	**54.8***	**13.3**	**13.5**	**14.5**	**15.1**	**13.6**	**15.1**
SD	**8.3**	**1.6**	**3.2**	**2.3**	**2.6**	**3.1**	**3.7**

*** - Significant difference between 0-ppm Ca and all other groups, $p < 0.0001$ (Tukey multiple comparison index), between other groups differences were not significant at the 5% level.**

Table 2.5 Percentage difference of mineral loss as measured by TMR

Tooth number	Group						
	Calcium concentration (ppm)						Natural saliva
	0	50	100	250	500	1000	
1	-290.2	16.1	22.0	1.1	27.5	25.2	41.3
2	-237.3	1.1	2.0	40.4	19.0	8.4	17.3
3	-309.7	11.1	6.0	22.0	29.1	9.4	3.0
4	-612.5	16.1	46.1	4.6	13.0	16.0	23.5
5	-259.9	21.5	-0.4	29.4	4.5	30.1	-10.8
6	-538.2	-2.0	5.2	13.5	17.5	15.7	11.2
7	-469.8	8.5	18.8	0.1	-0.7	13.0	6.9
8	-851.3	5.3	2.9	21.7	19.4	18.9	31.0
9	-326.4	3.9	8.2	23.3	17.5	8.6	41.7
Mean	**-432.8***	**9.1**	**12.3**	**17.3**	**16.3**	**16.2**	**18.3**
SD	**204.6**	**7.8**	**14.8**	**13.7**	**9.7**	**7.5**	**17.8**

*** - Significant difference between 0-ppm Ca and all other groups, $p < 0.0001$ (Tukey multiple comparison index), between other groups differences were not significant at the 5% level.**

"-" figures in this table mean that further demineralisation occurred in corresponding samples, while "+" figures point to mineral gain thus possible remineralisation.

Table 2.6 Percentage difference lesion depth as measured by TMR

Tooth number	Group						
	Calcium concentration (ppm)						Natural saliva
	0	50	100	250	500	1000	
1	-241.6	-4.6	2.5	18.1	6.1	14.8	12.6
2	-23.6	17.0	4.8	16.4	1.7	-15.0	-21.7
3	-196.6	3.5	13.2	-15.9	18.7	-11.3	-3.9
4	-488.9	19.4	48.2	-27.0	-27.0	3.0	12.6
5	-215.2	0.8	9.1	-5.8	5.1	-41.3	-18.3
6	-421.4	-11.6	29.4	2.7	12.2	28.4	6.8
7	-285.1	15.9	0.0	-21.2	-15.9	-16.9	11.8
8	-542.7	-33.6	-12.9	4.4	8.8	0.7	-22.7
9	-246.9	-5.2	20.8	3.2	-1.2	-21.0	20.8
Mean	**-295.8***	**0.2**	**12.8**	**-2.8**	**1.0**	**-6.5**	**-0.2**
SD	**162.0**	**16.7**	**18.0**	**15.9**	**14.2**	**20.7**	**16.9**

*** - Significant difference between 0-ppm Ca and all other groups, $p < 0.0001$ (Tukey multiple comparison index), between other groups differences were not significant at the 5% level.**

"-" figures in this table mean that further demineralisation occurred in corresponding samples, while "+" figures point to mineral gain thus possible remineralisation.

Figure 2.10 shows a histogram of the changes in mineral loss before and after the samples had been exposed to the experimental solutions. The 0-ppm Ca group shows that following exposure to the experimental solution significant ($p < 0.0001$) mineral loss occurred. Significant differences were observed between samples exposed to 0 ppm calcium and those subjected to calcium at concentrations between 50 ppm and 1000 ppm or calcium present in natural saliva.

Changes in lesion depth are shown histographically in Figure 2.11 and demonstrate a similar pattern to Figure 2.10.

Figure 2.12 illustrates the percentage changes in mineral loss and lesion depth after treatment of the specimens with the experimental solutions. In the absence of calcium, the percentage changes in both parameters were significantly different ($p < 0.001$) to those observed in the presence of remineralising solutions containing calcium between 50 and 1000 ppm.

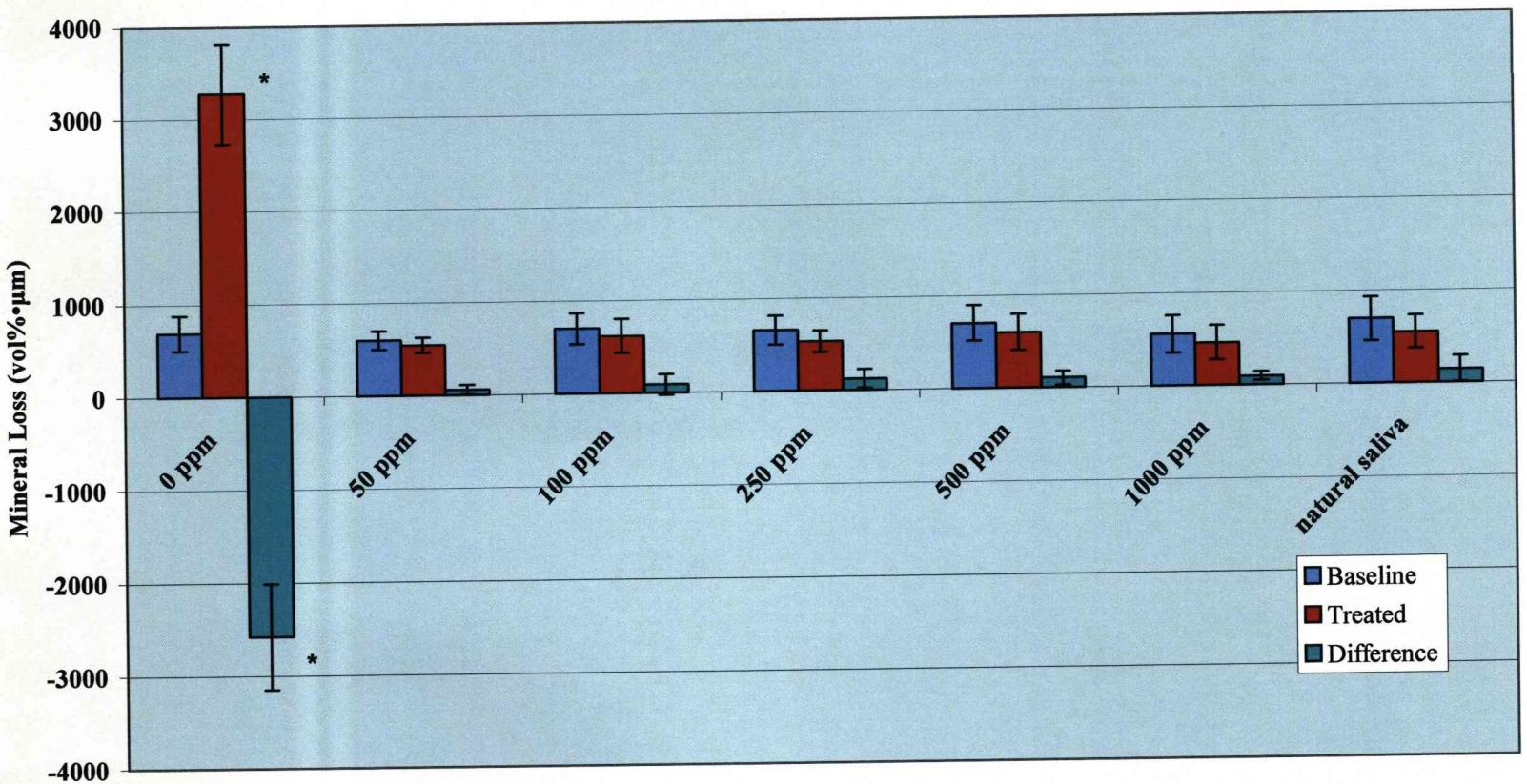


Figure 2.10 Diagram illustrating the changes of mineral loss before and after remineralisation (* - $p < 0.0001$)

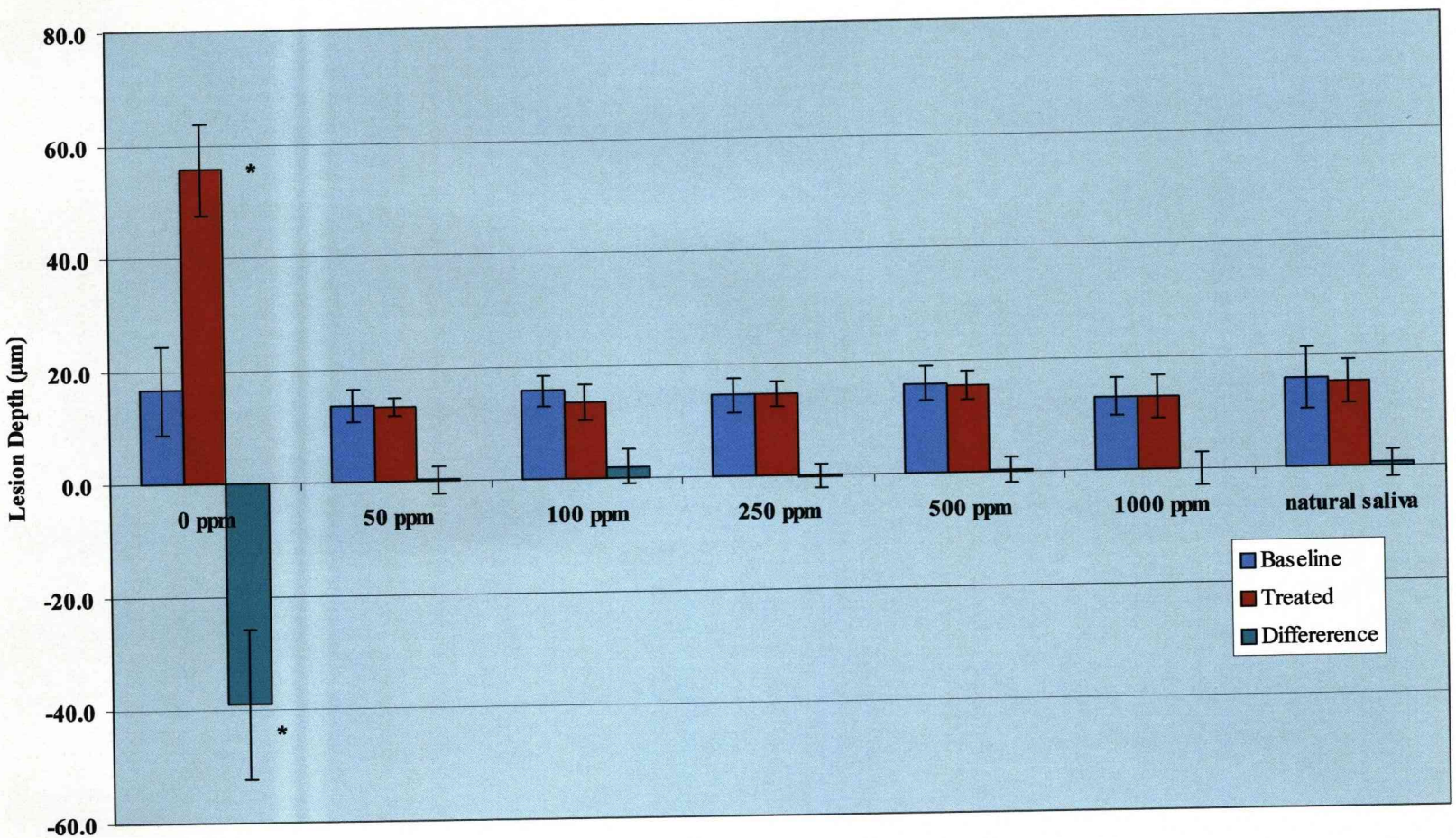


Figure 2.11 Diagram illustrating the changes of lesions depth before and after remineralisation (* - $p < 0.0001$)

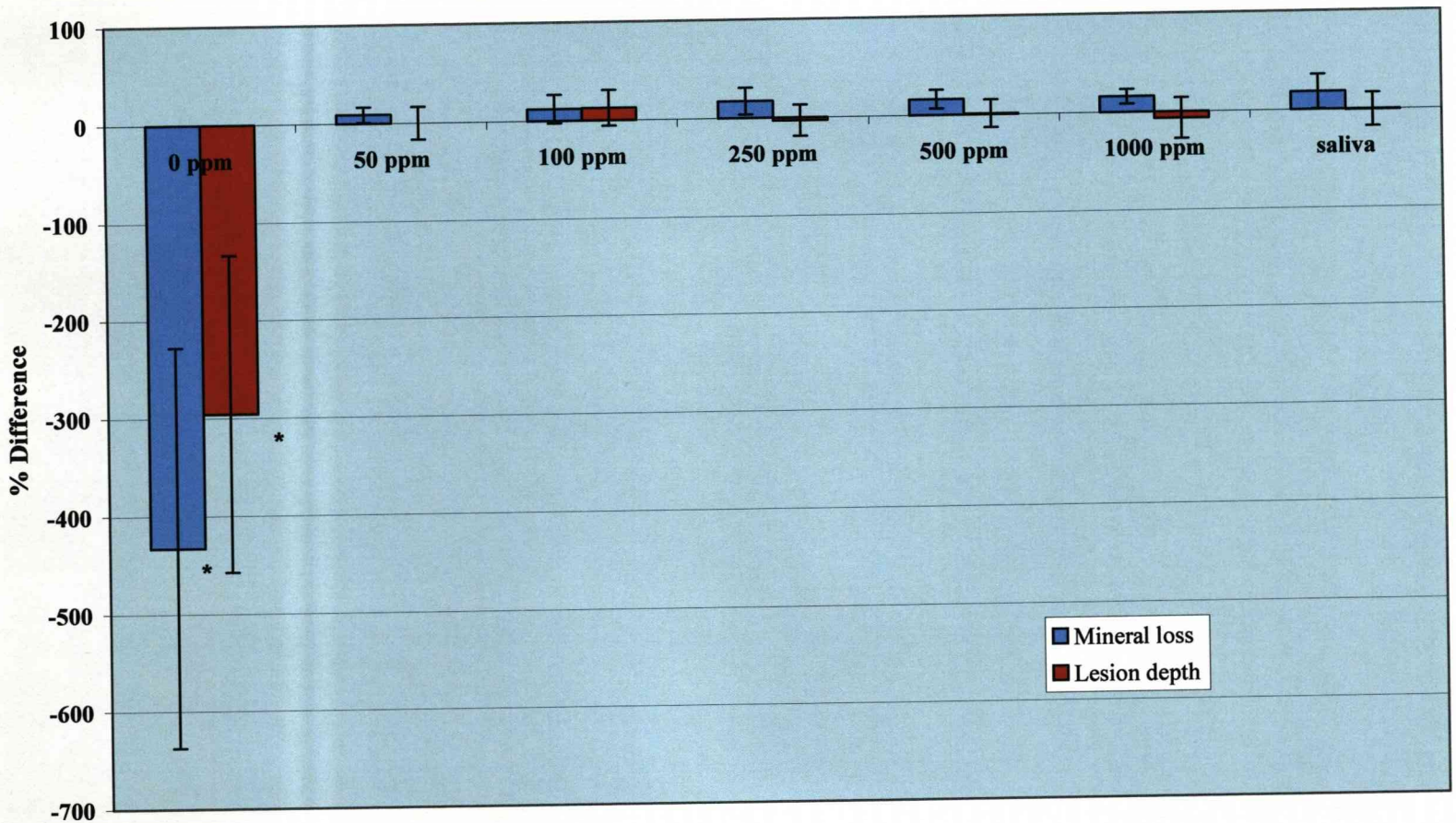


Figure 2.12 Diagram illustrating the percentage changes of mineral loss and lesions depth after remineralisation (* - $p < 0.0001$)

2.5 Discussion

In this chapter the possible remineralising effect of a specially formulated solution containing varying degrees of calcium lactate on pre-eroded human molar teeth was assessed *in vitro* against calcium-free solution and centrifuged natural human saliva.

The study showed that constantly surrounding artificially produced eroded enamel lesions *in vitro* with calcium containing remineralising solution promoted remineralisation of the experimentally softened surface.

Calcium is one of the major structural elements of tooth enamel, and is a key component of hydroxyapatites and it is also present in soluble form in saliva at hypersaturated concentrations between 0.75 and 1.75 mmol/L (Larsen and Fejerskov, 1989). Thus, calcium forms a natural universal ion-exchanging repair system which operates in the oral cavity and serves to protect teeth against attacks by intrinsic and extrinsic acids (Edgar *et al*, 1994). Dental erosion is caused by low pH (in the range 1-4) acids coming into direct contact with mineralised tooth surfaces, which act to dissolve the dental enamel layer by layer, which contrasts with the deeper sub-surface penetration seen during caries attack (Bartlett and Smith, 2000). When the erosive challenge is relatively small, calcium and phosphates present in saliva are usually able to restore the damaged surface crystals of calcium hydroxyapatite (Zero, 1996; Bartlett, 2006). In cases of persistent acid attacks (e.g. frequent vomiting episodes, frequent consumption

of acidic drinks and food) the balance shifts to favour further demineralisation of tooth enamel thus forming progressive lesions of dental erosion on affected teeth (Bartlett, 2006). In these circumstances intervention may be required to tip the process in favour of remineralisation, for example, by means of therapeutic and preventive solutions which are designed to provide the benefits afforded by natural saliva.

In this study calcium was added to solutions as calcium lactate, which has non-toxic properties, is completely tasteless and quite soluble. These properties are important considerations if these solutions were to be used *in vivo* in the management of dental erosion for example in patients suffering from frequent gastroesophageal reflux (such as those with conditions like bulimia nervosa). In these patients their own saliva is unable to clear gastric acid from the oral cavity, buffer it's acidity and remineralise the softened tooth surface and therefore they may benefit from the use of a remineralising solution. Several studies have confirmed the safety and remineralising potential of calcium lactate, when used as an additive in food, acidic drinks and mouthrinses in dental caries and erosion *in vivo* research (Shrestha *et al*, 1982; van der Hoeven *et al*, 1989; Beiraghi *et al*, 1989; Kashket and Yaskell, 1997).

The study reported here also demonstrated that the remineralising effect of experimental solutions did not depend on the concentration of calcium in the range used in the experiment. All groups, containing between 50 and 1000 ppm of calcium showed the same degree of mineral gain after 28 days of cycling pre-

eroded samples (multiples comparisons $p > 0.05$). However, large standard deviations meant that although in the 50-ppm calcium group mineral gain was only around half that in the 1000-ppm calcium group and similarly half that in the natural saliva group, no statistically significant difference was observed ($p > 0.05$). It could be argued that the standard deviations obtained suggest that increasing the number of samples in the study may lead to greater separation between the effects of low and higher calcium concentrations. In some previous *in vitro* and *in vivo* studies mouthrinses containing different salts of calcium such as phosphate and others were used (van der Hoeven *et al*, 1989, Papadogiannis *et al*, 1991, Amaechi *et al*, 1998b, Pretty *et al*, 2003a).

The pH of the remineralising solution was prepared close to neutral (6.7) in contrast with some reported anticaries remineralisation mouthrinses, where the pH was made slightly more acidic e.g. pH = 6 (Arends *et al*, 1992). But in cases of dental erosion this characteristic is not favourable, as the increased acidity could promote further erosion clinically, particularly if more soluble dentine is exposed.

The gain in mineral increased with an increase in calcium concentration up to 250 ppm, thereafter less mineral was gained as calcium concentrations were increased. One possible explanation for this is the blockage of ionic channels caused by hardening of the superficial layer of softened enamel, thus preventing the remineralisation of the subsurface lesion (Arends *et al*, 1992). Another possible explanation is that there is a threshold level beyond which

damaged hydroxyapatite crystals at the lesion surface cannot be repaired by increasing calcium concentration in the remineralising solutions.

Further demineralisation of lesions in the 0-ppm calcium group could possibly be explained by the effects of mechanical wearing of the softened surface by the agitated solution coupled with the absence of calcium ions, which may create a driving force, leading to the further release of calcium ions from the enamel hydroxyapatite into solution in an attempt to restore equilibrium.

Although the natural saliva group demonstrated a greater effect on remineralisation of eroded samples than any of the artificially produced calcium containing solutions, the difference was not statistically significant ($p > 0.05$). However, this trend could be explained by natural saliva containing constituents other than the ones present in artificial solution. For example, the composition and quality of organic and non-organic components of such a complex biological fluid as natural stimulated saliva would be difficult to replicate artificially. In natural saliva there is a dynamic equilibrium with the main salivary minerals (calcium and phosphate) in a supersaturated state with respect to hydroxyapatite which could be considered to be more efficient at restoring the crystals damaged by erosion (ten Cate and Imfeld 1996). In addition, the natural buffering capacity, together with salivary mucins, produced after centrifugation could also play an important role in ionic exchange and controlling the pH on the surface of eroded enamel (ten Cate and Imfeld, 1996; Meurman and Frank, 1991).

2.6 Conclusion

The findings of this *in vitro* study suggest that calcium ions in the form of calcium lactate play a protective role on eroded human enamel lesions, when they are stored in remineralising solution in agitated conditions, while cycling of pre-eroded tooth samples in the absence of calcium caused further demineralisation. Additionally, the presence of calcium led to the remineralisation of softened enamel surfaces in all calcium containing groups studied, although the trend demonstrated that 250-ppm strong calcium solution produced the greatest remineralising effect and would be the concentration of choice for future related studies using remineralising solution.

Natural saliva used as a positive control produced the most effective remineralising and protective properties, when compared with calcium containing solutions, however the difference between them was not significant at 5% level.

Chapter 3

REMINERALISATION POTENTIAL OF SOLUTIONS WITH DIFFERENT CONCENTRATIONS OF FLUORIDE ON ERODED LESIONS

UNIVERSITY OF
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3.1 Introduction

The situation in the oral cavity, when saliva and plaque fluid are supersaturated with respect to phosphates and calcium, plays the major role in ion exchange between tooth enamel and oral fluids (Dowd, 1999; Larsen and Pearce, 2003). Due to high concentration of these ions in potential remineralising environments, early caries lesions and surface softening demineralisations can be arrested and often reversed both *in vivo* and *in vitro* (Backer Dirks, 1966; Eisenburger *et al*, 2001; Aoba, 2004).

The process of remineralisation of early stages of enamel demineralised lesions is the process of deposition and possible partial or full restoration of lost mineral constituents of tooth tissue. The main factors affecting remineralisation of dental erosion have been discussed in the introductory chapter of this thesis and this chapter focuses on the role of fluoride in this process.

Although calcium and phosphate ions, in the form of hydroxyapatite crystals, are the main structural chemical components of dental hard tissues (Robinson *et al*, 2000), other ions, primarily fluoride are believed to play a significant role in the solubility of enamel and dentine (Clarkson and McLoughlin, 2000; Wiegand and Attin, 2003). The presence of fluoride in saliva, usually in small concentrations, enhances the precipitation of calcium and phosphate into the surface layer of dental enamel thus forming fluorapatites and

significantly increasing the acid resistance of tooth structure (ten Cate, 1999). The main sources of salivary fluoride are dietary, drinking water and fluoride containing dentifrices. Other topical and systemic applications of fluoride containing agents, such as tablets, mouthrinses, varnishes and so on, can also add to the levels of fluoride in oral fluid (Lagerlöf and Oliveby, 1994). Some of these, especially those with high concentration of fluoride, can act as slow release reservoirs which lead to sustained higher intra-oral fluoride levels (Rolla and Saxegaard, 1990). The rate of remineralisation is not fully dependent on the intra-oral concentration of fluoride ions, as even small amounts in saliva and dental plaque are able to promote greater remineralisation compared with higher concentrations (ten Cate *et al*, 1995; Hicks *et al*, 2004). This could be explained by the fact that pores on the surface of enamel lesions are small and the rate of mineral migration into the lesion body could be thus limited (ten Cate and Arends, 1977). Remineralisation of the subsurface layer, could also be affected by a substantial increase in mineral deposition in the overlying surface layer thus reducing it's permeability to calcium and phosphate ions (Pearce *et al*, 1995; Larsen and Richards, 2001).

Caries and erosive lesions are thought to develop under different oral conditions. Caries requires the presence of dental plaque containing organic acids with pH 4-5 (resulting from the fermentation of carbohydrates by plaque microflora). In this situation plaque fluid is undersaturated with respect to hydroxyapatite and mineral is lost from the tooth, conversely fluorapatite is supersaturated and therefore precipitates in the presence of fluoride ions (Larsen,

1974; ten Cate and Duijsters, 1983a, b). It has been suggested in the case of dental erosion, where no dental plaque is present and the pH on the surface of tooth falls below 3, (as a result of the presence of acidic drinks/food or gastric fluids in the mouth), both hydroxyapatite and fluorapatite will be undersaturated and both mineral types may be lost from the tooth surface producing characteristic eroded lesions (ten Cate and Imfeld, 1996). This implies that adding fluoride ions into the erosive conditions described above may not lead to the formation of fluorapatite and therefore would be unlikely to slow down or prevent acidic demineralisation (ten Cate, 1999). However, alternative arguments have been proposed and in contradistinction it has been reported that fluoride is able to prevent dental erosion (Meurman and ten Cate, 1996).

In addition, considering the remineralisation of dental lesions, fluoride is believed to work not only as a direct remineralising agent, but as an accelerator of mineral precipitation and crystal growth (Aoba, 2004). Depending on concentration, fluoride can affect the kinetics of mineral deposition, and thus the characteristics of surface and subsurface enamel apatites (Aoba *et al*, 2003). Fluoride present in oral fluid can alter the rate of the deposition of minerals by reducing the solubility of precipitated fluorapatites, which may enhance the driving force for apatite formation without any effect on the concentration of common minerals in the oral fluid (ten Cate and Featherstone, 1991; Elliott, 1994).

The effect of the frequent application of low concentration of fluoride is believed to be greater than less frequent application of higher concentrations. This has been shown by studying the prevention of demineralisation and the process of the remineralisation of caries lesions (Arends and Christoffersen, 1990; Arends *et al*, 1992). It is possible that because of the different nature of dental erosion compared with the caries process that changes in fluoride concentration may act differently in the prevention of erosion as well as the remineralisation of eroded surfaces.

Given the potential for acidic drinks to cause dental erosion one approach to reduce enamel dissolution has been to add fluoride (Deery *et al*, 2000, Restarski *et al*, 1945a; Spencer and Ellis, 1950; Holloway *et al*, 1958; Gedalia *et al*, 1981; Sorvari *et al*, 1988). The positive effects of fluoride in this respect however, are limited because of the possibility of exceeding recommended limits for fluoride intake, especially in children which could lead to problems of dental fluorosis.

An alternative approach to managing dental erosion is to attempt to quickly restore the dental enamel weakened by acid attack before the softened surface is removed by mechanical forces, such as using routine toothbrushing (Gedalia *et al*, 1981; Sorvari *et al*, 1988), chewing hard food and mechanical friction of opposing teeth as well as soft oral tissues (tongue, lips and cheeks). Being able to determine the most effective concentrations of fluoride in remineralising media used both in the prevention and early treatment of erosion

is undoubtedly of great importance (Bartlett *et al*, 1994, Jaeggi and Lussi, 1999; Attin *et al*, 2000).

3.2 Aim and Objectives

3.2.1 Aim

The aim of the study described in this chapter was to assess the remineralising potential of a new formula of remineralising solution with varying concentrations of fluoride and with a fixed concentration of calcium and phosphate ions on artificially produced dental erosion *in vitro*.

3.2.2 Objectives

The objectives of this study were to:

- investigate the remineralising effect of sodium fluoride on artificially produced erosive lesions *in vitro*;
- establish the optimum concentration of fluoride ions in remineralising solution in protection and remineralisation of artificial erosion;
- compare the remineralising effect of newly formulated solutions, containing different concentrations of fluoride, with natural saliva on eroded lesions.

3.3 Materials and Methods

This part of the study used another group of 75 extracted human molars and the methods used in the preparation of samples and the production of experimental erosion from these were the same as those described in Chapter 2 (see above). The main difference in this aspect of the study compared with the previous series of experiments reported above was to investigate the effects of different levels of fluoride in the artificial calcium containing remineralising solution.

3.3.1 Artificial remineralising solution

The artificial remineralising solution was prepared as described in Chapter 2 using a modified formula based on that described by Amaechi *et al* (1998a). Again this had reduced amounts of sodium carboxymethyl cellulose to produce appropriate viscosity, together with 0.4 g/L; KCl, 0.625 g/L; $MgCl_2 \cdot H_2O$, 0.03 g/L; K_2HPO_4, 0.102 mg/L; KH_2PO_4, 0.041 mg/L; methyl-p-hydroxybenzoate, 2.0 g/L. This solution was further modified by the addition of calcium lactate to produce calcium ions at a concentration of 250 ppm, which related to the trend for optimum remineralisation seen in Chapter 2 of this thesis. This formed the stock solution of remineralising agent which was subsequently used to prepare six experimental solutions differing only in fluoride concentration. The first group had no added fluoride and served as a negative control. The remaining solutions had sodium fluoride added to give 100 ppm fluoride ions in the 2^{nd} group, 250 ppm in 3^{rd}, 500 ppm in 4^{th}, 1000 ppm in 5^{th} and 12,000 ppm of fluoride in 6^{th} group. The hydrogen ion concentration of each of the experimental solutions was adjusted to pH 6.7 using KOH. Fresh stimulated and centrifuged natural human saliva was used as a positive control as per Chapter 2 (see above).

The remainder of the experimental procedure involving the production of enamel samples, remineralising cycles, TMR analysis was as described in Chapter 2.

3.3.2 Statistical analysis

The statistical analysis of mineral loss and lesion depth was carried out using one-way ANOVA and multiple comparison Tukey index (a=0.05) with statistical package SSPS. The percentage change in mineral content and lesion depth of each lesion was calculated as follows:

$$\%\Delta Z = \frac{\Delta Z(control) - \Delta Z(test)}{\Delta Z(control)} \times 100\%$$

$$\%ld = \frac{ld(control) - ld(test)}{ld(control)} \times 100\%$$

3.4 Results

The mean pH of orange juice used to produce the initial eroded lesions was measured to be 3.58 ± 0.01.

During the process of cutting the specimens into sections, one slab from the 0-ppm Fluoride group was destroyed thus leaving only eleven samples in this group. The remaining groups had twelve tooth slabs each.

Table 3.1 shows the mineral loss (vol%·μm) and Table 3.2 shows the lesion depth (μm) of the studied control specimens allocated to the experimental groups. The mean mineral loss ranged from 724.3 ± 105.1 in the natural saliva group to 783.7 ± 127.1 vol%·μm in the 250 ppm F group. The mean lesion depth ranged from 17.0 ± 1.7 in the 1000 ppm F group to 18.0 ± 2.5 μm in the 0 ppm F group. Neither control mineral loss nor control lesion depth in the experimental groups differed significantly ($p > 0.05$).

After 28 days of exposure to the experimental remineralising solutions and natural saliva certain changes in the mineral loss and lesion of the depth of the specimens were observed, details of which are shown in Tables 3.3 and 3.4. The largest detected change in mineral loss was found in 1000 ppm F group, where an increase in mineral loss to 1137.7 ± 308.6 vol%·μm (Table 3.3) and an increase in lesion depth to 21.6 ± 3.4 μm was recorded. The second highest changes in mineral loss and lesion depth were observed in 12,000 ppm F group

with mean value of 829.9 ± 156.7 vol%·μm and 22.4 ± 4.0 μm respectively, which point to further mineral loss as well. The only significant difference (the Tukey multiple comparison index) in mineral loss ($p < 0.001$) was found between 1000 ppm F group and all other experimental groups (0, 100, 250, 500, 12,000-ppm F and natural saliva). For lesion depth a similar pattern was also recorded using the same statistical approach with the only significant difference ($p < 0.001$) observed in the 1000-ppm F group as well as in 12,000-ppm F groups in comparison with other experimental groups (0, 100, 250, 500-ppm F and natural saliva).

Table 3.5 shows the percentage difference in mineral loss for the experimental groups as measured by TMR. In samples with positive values, mineral gain was observed after 4 weeks of cycling in the study solutions, while negative figures indicated that further demineralisation occurred in corresponding specimens. In the 0, 1000 and 12,000-ppm F groups the majority of tooth slabs demonstrated further net loss of mineral tissue from pre-eroded lesions. The highest percentage difference mineral loss was detected in 1000 ppm F group with mean of -45.5 ± 30.5%, which was found to be significantly different ($p < 0.001$) from that observed in all other experimental groups, when the Tukey multiple comparison index was used. In the natural saliva group the highest mineral gain was observed with a mean of 14.9 ± 8.8%, but no significant differences were found, when compared with other groups with net mineral gain ($p > 0.05$). The highest increase in mineral content among artificial solutions groups was observed in the group, where 500 ppm of fluoride was

added with again no significant difference in comparison with other groups, which showed remineralisation of the eroded lesion.

Similarly the percentage difference in lesion depth shown in Table 3.6 was also observed to be significantly different in the 1000 ppm F group as well as in 12,000 ppm F group, when compared with all other experimental group. As was mentioned previously these two groups showed further mineral loss at the end of the study, while in 500 ppm F group the maximum mineral gain had a mean value of 12.2 ± 21.1 %. No significant differences ($p > 0.05$) were found between groups, where mineral gain was discovered.

Table 3.1 Control mineral loss, vol%·μm as measured by TMR

Tooth number	Group						
	Fluoride concentration (ppm)						Natural saliva
	0	100	250	500	1000	12,000	
1	897.3	508.1	653.4	500.6	584.3	692.8	597.1
2	460.3	991.4	688.5	1087.8	945.4	560.9	694.8
3	560.9	694.8	808.0	764.0	804.2	821.8	857.6
4	1077.6	740.2	1036.6	736.9	822.4	734.7	715.5
5	736.9	732.5	859.9	804.2	903.2	906.8	710.6
6	849.9	833.8	703.4	881.2	746.1	790.6	811.0
7	653.4	709.9	974.8	923.7	892.0	709.9	750.6
8	786.6	603.0	879.3	681.4	585.2	656.5	836.5
9	556.5	703.4	716.5	700.1	716.4	603.0	585.2
10	717.2	583.0	716.4	897.6	920.0	836.5	814.9
11	679.3	879.3	687.9	777.0	780.3	897.6	780.3
12		718.5	679.3	523.2	630.1	925.9	537.1
Mean	**725.1**	**724.8**	**783.7**	**773.1**	**777.5**	**761.4**	**724.3**
SD	**175.0**	**131.6**	**127.1**	**165.6**	**128.3**	**121.1**	**105.1**

Unless marked, differences were not significant between groups at the 5% level.

Table 3.2 Control lesion depth, μm as measured by TMR

Tooth number	Group						
	Fluoride concentration (ppm)						Natural saliva
	0	100	250	500	1000	12,000	
1	19.5	17.9	15.3	11.3	15.1	15.6	15.3
2	19.4	20.9	16.8	22.0	19.7	15.7	16.7
3	15.7	16.7	20.0	14.0	16.7	15.1	22.6
4	23.4	18.6	20.3	20.1	17.1	16.2	18.3
5	20.1	15.3	18.4	16.7	18.3	17.6	15.4
6	17.4	17.0	16.1	17.8	17.1	17.5	19.8
7	15.3	18.8	18.6	19.0	19.1	18.8	17.7
8	18.0	17.8	17.5	20.2	14.2	17.3	17.8
9	16.1	16.1	17.5	17.9	16.7	17.8	14.2
10	15.5	15.9	16.7	22.0	17.4	17.8	18.1
11	17.6	17.5	15.9	14.9	18.1	22.0	18.1
12		17.2	17.6	13.1	14.5	23.1	14.3
Mean	**18.0**	**17.5**	**17.6**	**17.4**	**17.0**	**17.9**	**17.4**
SD	**2.5**	**1.5**	**1.6**	**3.5**	**1.7**	**2.5**	**2.4**

Unless marked, differences were not significant between groups at the 5% level.

Table 3.3 Final mineral loss, vol%·μm as measured by TMR

Tooth number	Group						
	Fluoride concentration (ppm)						Natural saliva
	0	100	250	500	1000	12,000	
1	840.0	410.6	616.4	575.0	534.6	741.8	521.3
2	713.0	837.9	572.9	929.4	1093.5	852.9	564.0
3	669.4	580.0	717.7	645.4	1394.7	698.4	656.2
4	1117.0	716.2	1028.4	505.2	868.4	1000.2	644.8
5	710.9	687.1	878.0	478.4	1398.7	1134.7	766.0
6	569.3	785.5	626.8	734.8	1216.2	955.3	639.7
7	694.7	606.3	868.1	638.5	1537.6	915.7	580.1
8	767.2	649.2	739.7	647.8	756.4	1038.0	704.7
9	618.8	570.7	705.4	701.2	1413.6	649.0	504.8
10	778.7	480.1	620.6	925.4	1345.9	807.5	623.2
11	797.9	938.6	664.4	626.0	1164.5	825.4	662.3
12		825.6	647.4	647.4	928.6	1095.6	487.7
Mean	**752.5**	**674.0**	**723.8**	**671.2**	**1137.7***	**892.9**	**612.9**
SD	**144.4**	**155.5**	**135.4**	**139.8**	**308.6**	**156.7**	**83.8**

*** - Significant difference between 1000-ppm F and all other groups, $p < 0.001$ (Tukey multiple comparison index), between other groups differences were not significant at the 5% level.**

Table 3.4 Final lesion depth, μm as measured by TMR

Tooth number	Group						
	Fluoride concentration (ppm)						Natural saliva
	0	100	250	500	1000	12,000	
1	19.8	20.8	15.6	14.5	19.0	18.3	13.3
2	16.4	17.6	15.7	22.0	21.3	25.0	12.6
3	15.5	12.7	17.1	17.1	24.2	16.0	19.4
4	27.4	15.9	19.7	12.9	23.4	22.3	15.7
5	14.8	11.5	19.2	11.0	22.3	26.2	21.1
6	14.5	17.7	14.3	13.9	20.5	24.4	15.1
7	16.9	19.8	17.2	13.2	26.6	24.6	15.4
8	17.7	14.5	16.9	15.7	13.6	19.2	19.5
9	18.8	12.8	16.6	16.3	24.6	20.2	11.9
10	16.0	12.7	14.8	16.4	22.7	18.3	17.2
11	18.7	18.4	16.0	12.0	22.3	24.4	19.6
12		18.2	13.9	12.6	19.2	29.4	10.8
Mean	**17.9**	**16.0**	**16.4**	**14.8**	**21.6***	**22.4***	**16.0**
SD	**3.6**	**3.1**	**1.8**	**3.0**	**3.4**	**4.0**	**3.4**

*** - Significant difference between 1000-ppm and 12,000-ppm F and all other groups, $p < 0.001$ (Tukey multiple comparison index), between other groups differences were not significant at the 5% level.**

Table 3.5 Percentage difference of mineral loss as measured by TMR

Tooth number	Group						
	Fluoride concentration (ppm)						Natural saliva
	0	100	250	500	1000	12,000	
1	6.4	19.2	5.7	-14.9	8.5	-7.1	12.7
2	-54.9	15.5	16.8	14.6	-15.7	-52.1	18.8
3	-19.4	16.5	11.2	15.5	-73.4	15.0	23.5
4	-3.7	3.2	0.8	31.4	-5.6	-36.1	9.9
5	3.5	6.2	-2.1	40.5	-54.9	-25.1	-7.8
6	33.0	5.8	10.9	16.6	-63.0	-20.8	21.1
7	-6.3	14.6	11.0	30.9	-72.4	-29.0	22.7
8	2.5	-7.7	15.9	4.9	-29.2	-58.1	15.8
9	-11.2	18.9	1.5	-0.2	-97.3	-7.6	13.7
10	-8.6	17.7	13.4	-3.1	-46.3	3.5	23.5
11	-17.5	-6.7	3.4	19.4	-49.2	8.0	15.1
12		-14.9	4.7	-23.7	-47.4	-18.3	9.2
Mean	**-6.9**	**7.4**	**7.8**	**11.0**	**-45.5***	**-19.0**	**14.9**
SD	**21.4**	**11.8**	**6.1**	**19.2**	**30.5**	**22.8**	**8.8**

*** - Significant difference between 1000-ppm F and all other groups, $p < 0.001$ (Tukey multiple comparison index), between other groups differences were not significant at the 5% level.**

"-" figures in this table mean that further demineralisation occurred in corresponding samples, while "+" figures point to mineral gain thus possible remineralisation.

Table 3.6 Percentage difference lesion depth as measured by TMR

Tooth number	Group						
	Fluoride concentration (ppm)						Natural saliva
	0	100	250	500	1000	12,000	
1	-1.5	-16.2	-2.0	-28.6	-25.8	-17.6	12.9
2	15.5	15.4	6.5	0.0	-8.2	-59.0	24.6
3	1.4	24.1	14.8	-22.1	-44.9	-5.6	14.4
4	-17.1	14.7	3.1	36.1	-36.6	-37.7	14.1
5	26.2	24.6	-4.3	34.4	-21.9	-48.9	-36.8
6	16.7	-4.1	11.5	21.9	-19.7	-39.6	23.6
7	-10.5	-5.6	7.5	30.4	-39.4	-30.6	13.0
8	1.7	18.5	3.4	22.5	3.9	-11.2	-9.7
9	-16.8	20.7	5.1	8.6	-47.0	-13.3	16.2
10	-3.2	20.1	11.7	25.3	-30.5	-3.0	5.0
11	-6.2	-5.4	-0.6	19.5	-23.5	-11.1	-8.0
12		-5.6	21.0	4.2	-32.7	-27.1	24.5
Mean	**0.6**	**8.4**	**6.5**	**12.7**	**-27.2***	**-25.4***	**7.8**
SD	**13.9**	**14.6**	**8.9**	**21.1**	**14.8**	**18.0**	**18.0**

*** - Significant difference between 1000-ppm and 12,000-ppm F and all other groups, $p < 0.001$ (Tukey multiple comparison index), between other groups differences were not significant at the 5% level.**

"-" figures in this table mean that further demineralisation occurred in corresponding samples, while "+" figures point to mineral gain thus possible remineralisation.

Figure 3.1 shows a histogram of the changes in mineral loss before and after the specimens were exposed to the experimental solutions. In groups with high fluoride concentration (1000 and 12,000 ppm F) further demineralisation of pre-eroded lesions was noticed ($p<0.001$). The difference between the control and experimental values was also found to be significantly greater in these groups. Multiple comparisons show significant differences ($p<0.001$) between the 1000-ppm F group and all other groups and in comparison between the 12,000-ppm F group and the other experimental groups ($p<0.05$).

Figure 3.2 demonstrates a similar pattern of significant differences in lesion depth changes as in Figure 3.1.

Figure 3.3 illustrates the percentage changes in mineral loss and lesion depth after treatment of the studied samples with the experimental solutions. Adding fluoride in high concentrations prevented the damaged surface of the artificial eroded lesions remineralising in the presence of calcium and phosphate. The 0-ppm fluoride group also showed further mineral loss, but the difference was not significant in comparison with mineral gain groups. The highest remineralisation according to the mineral loss was observed in natural saliva followed by the 500-ppm fluoride group with no statistical difference between them and other groups with respect to mineral gain (100, 250-ppm F).

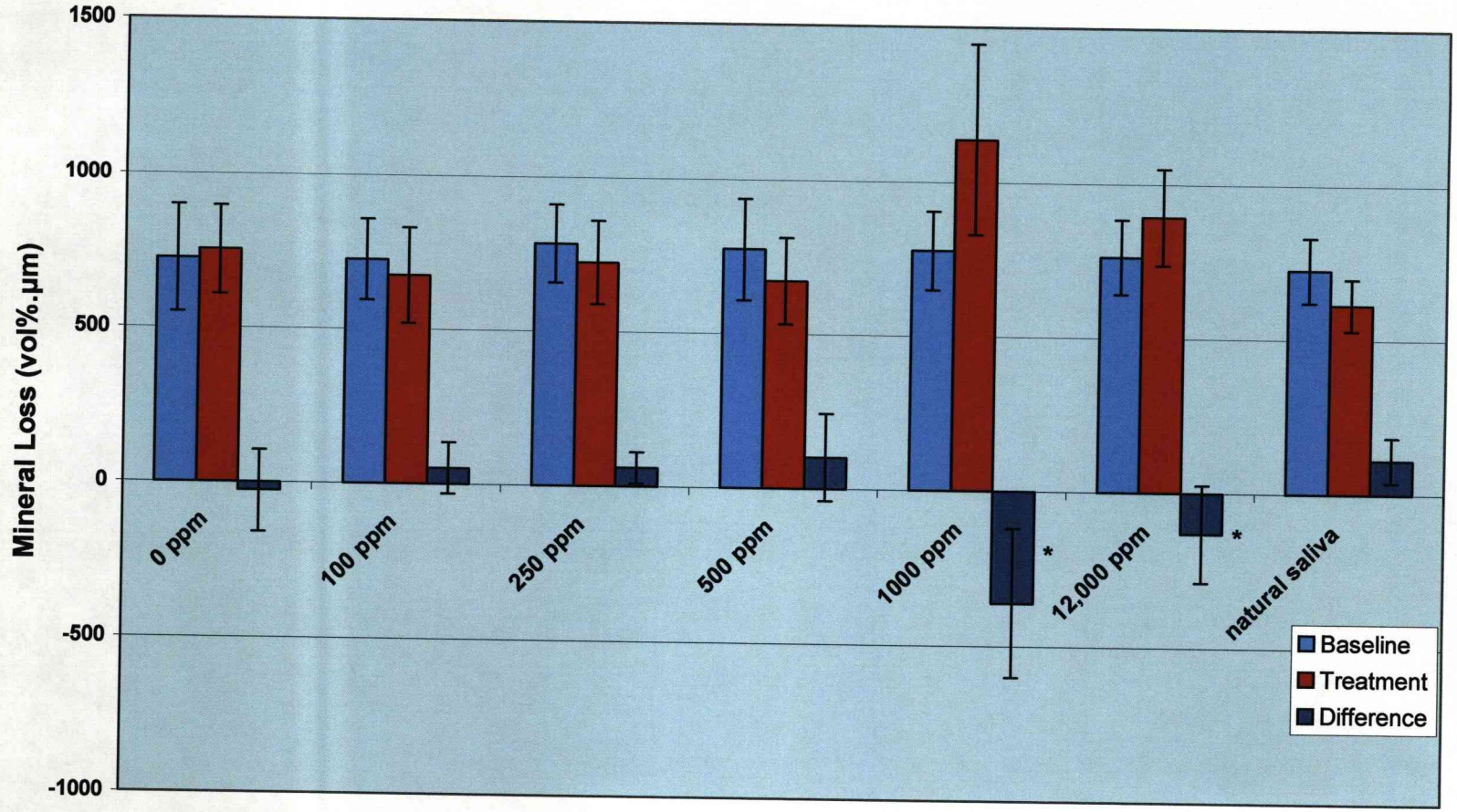

Figure 3.1 Diagram illustrating the changes of mineral loss before and after remineralisation (* - $p < 0.001$)

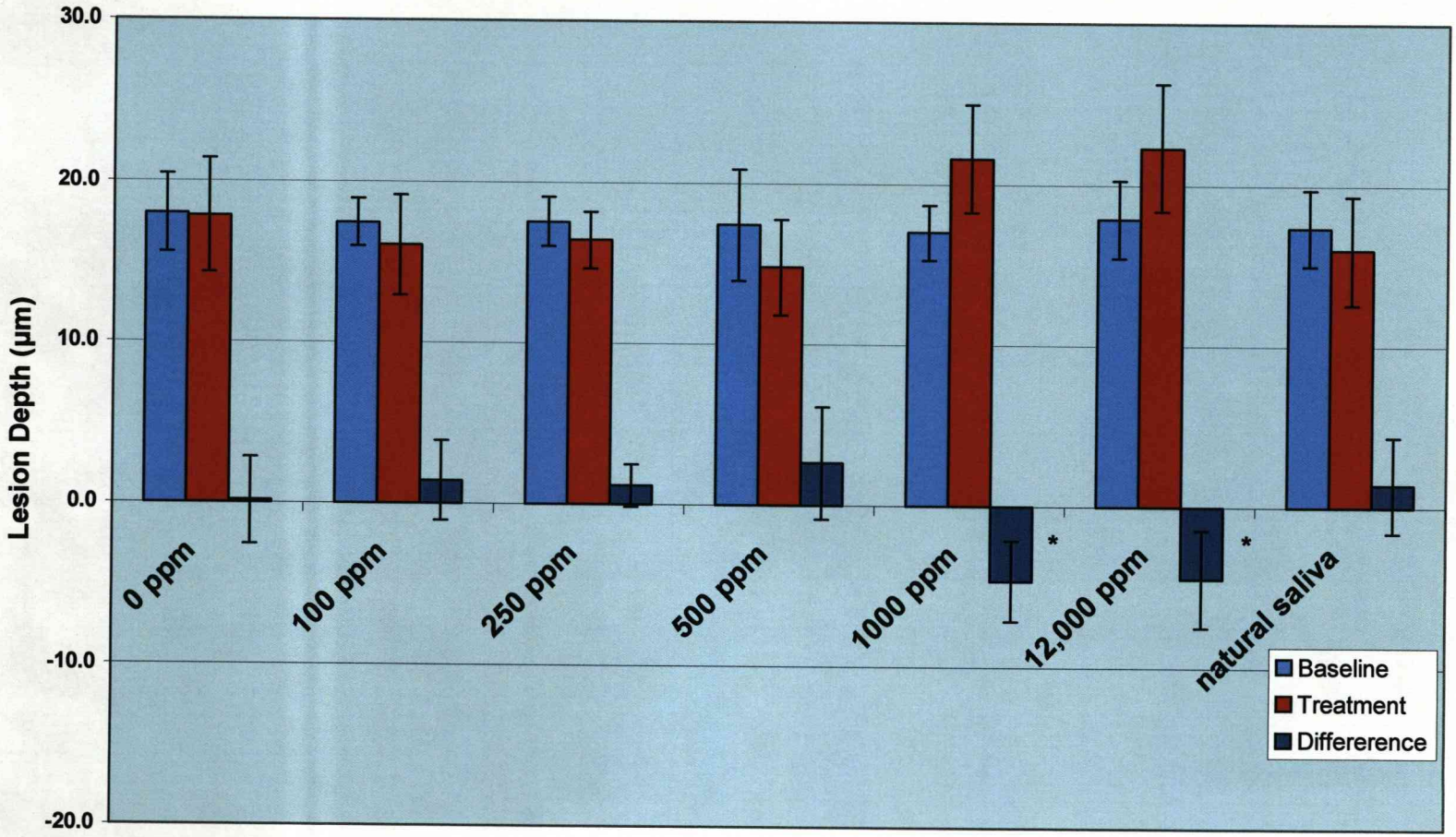

Figure 3.2 Diagram illustrating the changes of lesions depth before and after remineralisation (* - $p < 0.001$)

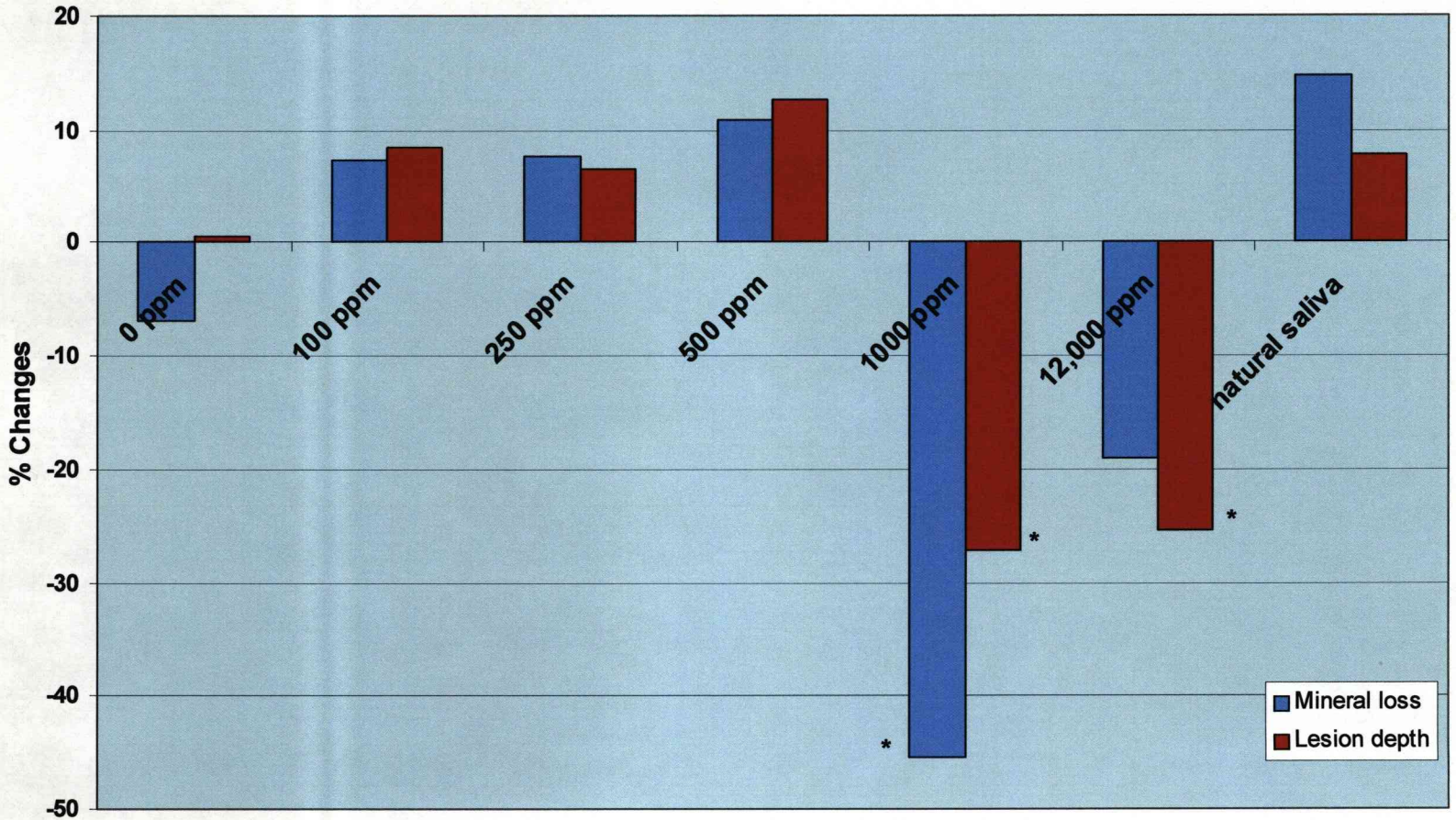

Figure 3.3 Diagram illustrating the percentage changes of mineral loss and lesions depth after remineralisation (* - $p < 0.001$)

3.5 Discussion

As discussed in previous chapters abnormal toothwear can be found in patients suffering with such intrinsic conditions as anorexia nervosa and bulimia nervosa (Hellström, 1977; Milosevic and Slade, 1989), gastro-oesophageal reflux (Barlett *et al*, 1996), rumination (Gilmour and Beckett, 1993). In this group of individuals symptomatic or often voluntary regurgitation or vomiting can force highly acidic gastric contents to appear in the mouth. The effect of the erosive challenge on dental enamel can be exaggerated by the reduction of the secretion of saliva, which may be the result of a number of factors, e.g. drug induced xerostomia (Meurman *et al*, 1994), atrophic changes in salivary glands in anorexic patients (Hellström, 1977), as well as the uncontrolled use of diuretics and laxatives and also deliberate vomiting, which cause general dehydration (Clark, 1985). In such cases it is important to provide patients with means that could maximise the protection of their teeth against aggressive acidic environment and possibly restore to its natural hardness already softened dental enamel.

The use of saliva substitutes and remineralising solutions after vomiting episodes and regurgitation could provide remineralising ions as well as acting to dilute and clear acids from the teeth surfaces. The latter is critical in case of abrasion as patients with vomiting episodes tend to brush their teeth with a toothbrush thus completely removing the softened enamel, which exacerbates the

severity of dental erosion (Clark, 1985). The work by Attin *et al* (2000) demonstrated that a time gap of at least an hour should follow the erosive challenge to avoid this problem; hence a mouthrinse could have potential benefits in removing residues of gastric contents from the teeth in this window of opportunity. Enhancing the remineralising properties of such mouthrinses could have additional benefits for the prevention of more severe tooth erosion.

This chapter describes the effect of the varying concentration of sodium fluoride in a specially formulated remineralising solution (mouthrinse) on pre-eroded extracted human molar teeth under *in vitro* conditions. Fluoride-free solution and centrifuged natural human saliva were used as controls.

To overcome the effect of lower fluidity of rather viscous artificial salivas designed to mimic the natural conditions (Gelhard *et al*, 1983; Davies, 2000) and to make the remineralising solution more effective in removing the remains of highly acidic gastric contents, the viscosity of the remineralising artificial saliva was reduced by significantly decreasing the amount of carboxymethyl cellulose added (Amaechi *et al,* 1998a). The resulting composition should have better organoleptic and cleaning properties during its use *in vivo* (Arends *et al*, 1992).

The study showed that four-week cycling of artificially produced eroded enamel lesions in solutions containing all necessary major mineral components led to a marginal remineralisation of experimentally softened surface in the presence of moderate concentrations of sodium fluoride as well as in natural saliva, while further demineralisation was noticed in the presence of high and

very high fluoride concentrations. The remineralising solution without added fluoride also showed a slight decrease in mineral content of studied samples, however this difference was not found to be statistically significant at the 5% level.

The data demonstrated that the remineralising effect of experimental solutions did not seem to depend on the concentration of fluoride in the range used in the experiment. There were relatively large standard deviations observed within the data, but this is to be expected as the data reflects variations in biological specimens, rather than duplicated samples.

There are a number of recent papers that report that fluoride has a 'protective' or 'promoting' effect, to a lesser or greater extent (depending on concentrations), rather than a 'remineralising' effect *per se*. Larsen (2001) reported that the erosion-preventive effect of 'even high fluoride concentrations' is limited, when considering the erosive potential of orange juice with or without the addition of fluoride (as calcium fluoride, however). More significantly, perhaps, Larsen and Richards (2002) went on to publish a paper entitled, 'Fluoride is unable to reduce dental erosion from soft drinks'. On the contrary further evidence, as to fluoride's anti-erosive properties have been produced, van Rijkom *et al* (2003) reported that topical titanium fluoride application provided a potential treatment option in erosion prevention (more so than for sodium fluoride). Using profilometry, Hughes *et al* (2004) showed that fluoride applied to enamel either in acidic conditions or as a pre-treatment reduced enamel

erosion *in vitro*. Ganss *et al* (2004) showed that intensive fluoride treatments of erosive lesions *in situ* prevented significant erosion lesions' progression – even under relatively aggressive erosive conditions. In an *in vivo* study, Young *et al* (2006) showed that stannous fluoride dentifrice reduced erosion-like lesions' demineralisation.

There is, however, little clear evidence in the literature showing that fluoride has the potential to actively remineralise erosion lesions *per se*, as opposed to *preventing* erosion of reducing erosion-related dissolution.

One of the possible explanations of further loss of minerals from eroded lesions in the presence of very high fluoride concentrations (1,000 and 12,000 ppm) could be the effect of formation of calcium fluoride precipitates on the surface of softened enamel blocking or preventing the incorporation of calcium and phosphates in the net enamel apatites in the process of nucleation and crystal growth, which is important in remineralisation (Arends *et al*, 1992). At the same time the samples were subject to strong continuous agitation in solution, which could have different mechanical and micromechanical effect of tooth surface, when either apatite crystal growth occurs or the precipitation of calcium phosphate prevails.

The other explanation of the unexpected effect of high fluoride concentrations might be related to the time and/or frequency of the contact between the solution and lesions. There was evidence reported by Lammers *et al* (1989) that constant presence of demineralised teeth in remineralising solution

containing fluoride at 2 ppm may inhibit or fail to stimulate remineralisation. As that study was performed on caries lesions the chemistry of fluoride influence on surface eroded lesions could had a similar effect but to a different extent at different concentrations of fluoride in remineralising solutions.

3.6 Conclusion

The results from this *in vitro* study suggest that artificial remineralising solutions containing calcium, phosphate and a moderate level of fluoride has a protective and remineralising effect on artificially formed eroded lesion in human teeth. There was, however, no clear relationship between increasing the concentration of calcium or fluoride and the remineralising effect observed.

The data analysed suggest that further studies are necessary to investigate the effect of mechanical agitation on softened surfaces in pH neutral conditions as well as the influence of high and very high fluoride concentrations on remineralisation of eroded lesions *in vitro*.

The findings from two laboratory studies described in this and previous chapters suggest the use of 250 ppm calcium in the form of calcium lactate and 500 ppm fluoride in the form of sodium fluoride for producing the artificial remineralising solutions for future work *in vitro* and in clinical trials performed *in vivo* or *in situ* to further investigate the problem of dental erosion, prevention and treatment of this condition.

Chapter 4

EVALUATION OF DENTAL EROSION USING QUANTITATIVE LIGHT-INDUCED FLUORESCENCE

UNIVERSITY OF
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4.1 Introduction

Following several scientific reports conducted through the 1980's and into the early 1990's, which studied the effect of auto-fluorescence of dental enamel and dentine, together with changes in their optical properties during early demineralisation (Bjelkhagen and Sundström, 1981; Bjelkhagen *et al*, 1982; Sundström *et al*, 1985; Hafström-Björkman *et al*, 1992.), intensive research has been carried out into the relationship between the mineral content of dental tissues and their optical properties *in vitro* (Emami *et al*, 1996; Hall *et al*, 1997b; Ando *et al*, 1997), *in situ* (Al-Khateeb *et al*, 1997) and *in vivo* (de Josselin de Jong, 1995; Al-Khateeb *et al*, 1998; Tranæus *et al*, 2001). As a result laser and light fluorescence has been shown to be able to quantify the amount of mineral loss or gain in early caries lesions in both natural and artificially developed environments.

In the technique known as Quantitative Light-induced Fluorescence illuminating the tooth with either an argon laser or filtered xenon light leads to its scattering in dental enamel thus allowing changes in the mineral content to be visualised (ten Bosch, 1996). This area appears as a dark spot. By measuring the scattering of fluorescence radiance it is possible to relate this phenomenon to the amount of mineral leaving or entering the demineralised area (Rousseau *et al*, 2002), which is well correlated with transverse microradiography (TMR) the accepted 'gold standard' for directly quantifying the tooth mineral changes (ten Bosch, 2000).

It is believed that dentinal tissue fluoresces significantly more and probably contributes to the whole fluorescence of tooth to a greater degree than enamel alone, however the actual cause of the fluorescence radiance due the illumination of filtered blue light has not been answered completely, as it was shown (Rousseau *et al*, 2002) that the thickness of dentine and dentino-enamel junction itself do not reflect the degree of mineral loss in enamel. However, Ando *et al* (2003) demonstrated that the level of fluorescence radiance scattering occurred in the area of artificial caries lesion depends on the thickness of enamel, which was shown under illumination of 488-nm argon laser, so further research is required to fully understand the nature of dental fluorescence and its behaviour that results from the demineralisation processes. That said, it is still generally accepted that early changes in enamel induced by demineralisation process trigger off a different degree of back scattering of fluorescence radiance compared with sound tooth or area (ten Bosch, 2000, Angmar-Månsson and ten Bosch, 2001), which makes it possible to distinguish and quantify, subsequently relating it to a certain degree of mineral loss or gain by means of sophisticated computer software.

While the main application of QLF in dental research remains for *in vitro* and *in vivo* caries investigations and more recently for dental plaque and tooth colouring/staining the interest of researches to use this technique in studying dental erosion *in vitro* has remained relatively low and under explored.

As described previously artificial enamel erosive lesions start as initial surface softening, which if allowed to progress, consists of two distinctive areas: a

crater caused by the total loss of enamel and a layer of demineralised softened surface at the base of the crater (Figure 4.1). Due to such persistent forces like acid corrosion and mechanical agitation of erosive solutions the softened layer of enamel completely dissolves, increasing the depth of the crater and revealing the underlying enamel making it prone to further softening (Amaechi and Higham, 2001). If the crater gradually deepens it might be possible for the degree of surface demineralisation to remain the same for erosive challenges occurring for different periods of time. Thus bearing in mind the currently accepted views on the nature of fluorescence of demineralised enamel (ten Bosch, 2000) and the effect of enamel thickness to calculate its mineral content (Ando *et al*, 2003) it is possible to hypothesise that QLF will not be able to measure accurately the progression of dental erosion. However, in a contrary view Pretty *et al* (2004) demonstrated the ability of QLF to detect and longitudinally monitor the development of dental erosion *in vitro*, in an experiment employing a constant erosive challenge of 15-hour acid attack. It has been shown that there is a strong positive correlation between enamel mineral loss measured by QLF (ΔQ) when compared with that measured by TMR (ΔZ). It has been argued that not only does the radiance scattering in the body of demineralised enamel affect the background fluorescence of the affected tooth, but also the walls of crater influence the optical properties of the eroded lesion. In the model suggested to explain how QLF might work in eroded enamel it is hypothesised that the walls of the eroded crater lead to extra scattering within the lesion, generating a shadowing effect and producing loss of fluorescence correlating with time of the progression of the lesion (Pretty *et al*,

2004). Transverse microradiography an established standard technique in quantifying mineral loss and gain both *in vitro* and *in vivo* is widely employed in analysis of dental caries (de Josselin de Jong *et al*, 1987) and erosion studies (Amaechi *et al*, 1998a). It is a highly accurate and reliable system and is widely regarded as the gold standard for this type of evaluation, but it is not suitable for longitudinal studies, as it requires the destruction of the specimens, it is also time consuming and unsuitable for use in *in vivo* experiments. The increasing interest in studying dental erosion resulting from the rise in prevalence of toothwear in the population has driven the need to find a suitable method that could be used for evaluating dental erosion which could not be met by TMR. It is possible that a technique based on changes in enamel fluorescence, such as QLF, could be the answer to these demands.

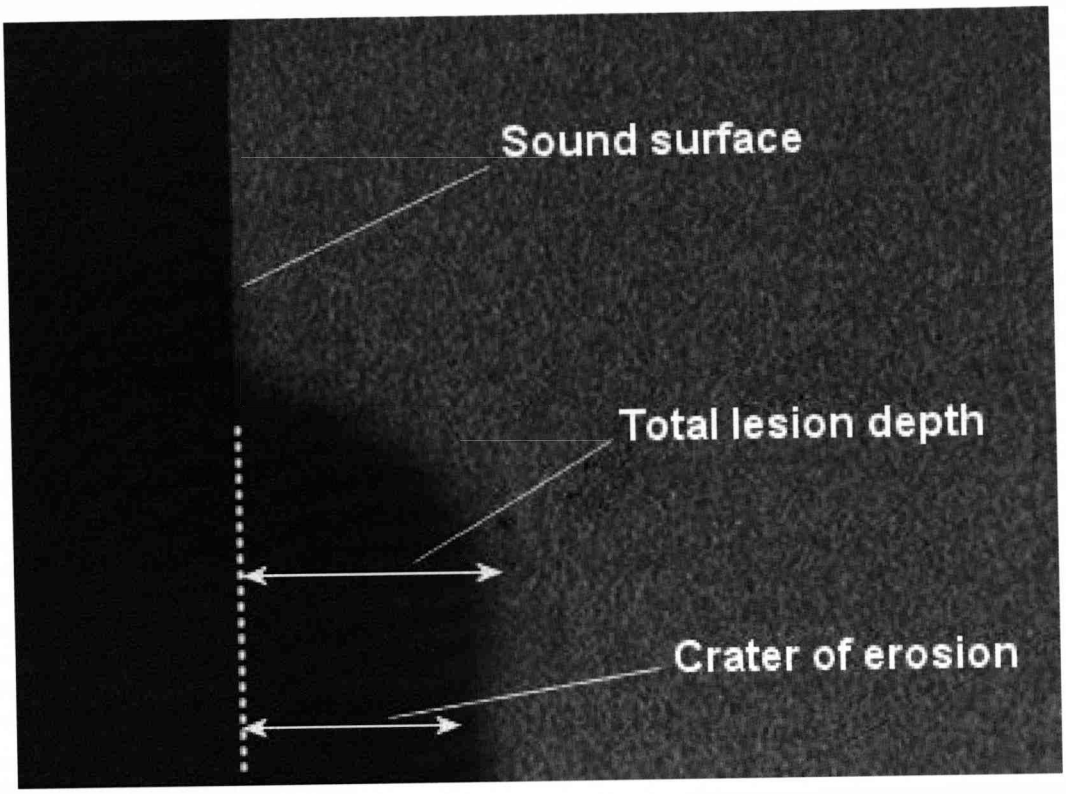


Figure 4.1 TMR image showing a section through eroded enamel

The development of large community dental preventive programs, the wider use of fluoridated dentifrices, and trends towards more preventive dental practice have resulted in fewer teeth being extracted. Increasing concerns relating to ethical issues involved in collecting and using human tissues in research, make the sourcing of human teeth for dental research particularly ones free from dental caries, a growing obstacle and investigators have turned to the use of tooth specimens from other mammals, especially from cattle (Pearce, 1983; Arends *et al*, 1989; Amaechi, *et al*, 1998c). Bovine teeth are readily available in large numbers,

with significantly fewer variations due to relatively standardised populations and methods of husbandry, together with comparable homogenized ages of animals (now up to 3 years old) are making the research models more standardised and reproducible (Edmunds *et al*, 1988; Arends *et al*, 1989).

The macro and microscopic structure of bovine teeth are considered to be different from human in the arrangements and dimensions of prisms, interprismatic zone, size of crystallites, (Boyde, 1965; Arends and Jongebloed, 1978; Whittaker *et al*, 1983). The sound bovine teeth are more porous and more prone to be penetrated by acids during artificial caries challenge and dyes than human (Arends and Schuthof, 1980; Featherstone and Mellberg, 1981; Edmunds *et al*, 1988), however the density and mineral levels of bovine enamel as well as penetration in the areas with artificially produced caries lesions are similar to human enamel (Edmunds *et al*, 1988). Thus, despite bovine teeth having several advantages in dental research, it is important to recognise the differences that exist between human and bovine enamel when these tissues are used in experiments involving changes in mineralisation.

4.2 AIM AND OBJECTIVES

4.2.1 Aim

The aim of the current study was to evaluate the *in vitro* use of Quantitative Light-induced Fluorescence technology to study dental erosion.

4.2.2 Objectives

The objectives of this study were to:

1. - investigate the ability of QLF to detect and quantify dental erosion;
2. - correlate QLF with transverse microradiography (TMR);
3. - observe differences in the detection of dental erosion between bovine and human teeth;
4. - establish the potential advantages and disadvantages of using QLF compared with TMR.

4.3 MATERIALS AND METHODS

4.3.1 Enamel samples

4.3.1.1 Bovine enamel

25 bovine incisors from 6-week old calves were chosen for the study and were prepared as shown below. After examining these under light microscopy (Nikon SMZ-10, Japan) at magnification x3 for the presence of micro cracks and other defects thirteen incisors were selected to be used in the erosion experiment.

4.3.1.2 Human enamel

30 freshly extracted human molar teeth free from caries, enamel malformations and visible cracks were collected and prepared as shown below. After preparation the molars were examined using a light microscope (Nikon SMZ-10, Japan) and thirteen with no defects (cracks, white spots, damaged surface) buccally were selected for the study.

4.3.2. Preparation of Samples

The human and bovine teeth were then prepared as follows; all soft tissue was removed from the outer tooth surface using hand periodontal scalers, then the teeth were cleaned with a toothbrush with pumice and the buccal/labial surface was polished with 1,200-grit abrasive sandpaper (Wet or Dry Sandpaper, 151 Products Limited, Manchester, UK). The roots were cut off and each tooth crown was longitudinally sectioned into two unequal parts: approximately one third for QLF analysis and two thirds to be used for TMR analysis (Figure 4.2). The smaller tooth slabs were coated with non-fluorescent acid-resistant nail varnish (MaxFactor®, Procter and Gamble, Weybridge, UK) except for exposed windows approximately 4x4 mm on the buccal surfaces. The other slabs (for TMR) were painted with regular acid-resistant nail varnish (MaxFactor®, Procter and Gamble, Weybridge, UK) except for two exposed rectangular windows approximately 8x2 mm for human and 12x2 for bovine teeth on the buccal surfaces (Figure 4.2).

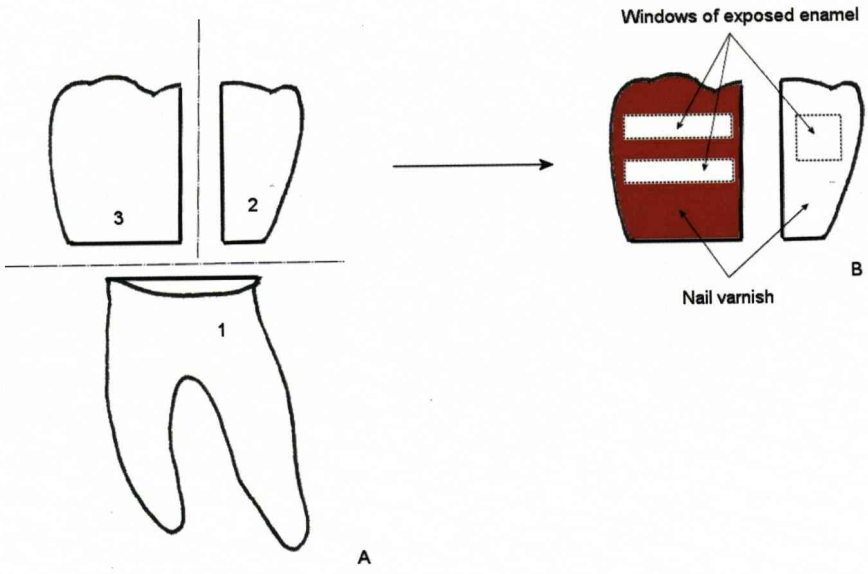


Figure 4.2 A&B – Diagrammatic representations illustrating how the experimental teeth were sectioned, and initial preparation prior to further sectioning

The tooth slabs selected for TMR analysis were cut into ten smaller slabs bearing exposed windows with nail varnish borders (Figure 4.3). The cut surfaces of these tooth blocks were covered with corresponding nail varnish to avoid acidic dissolution of underling enamel and dentine.

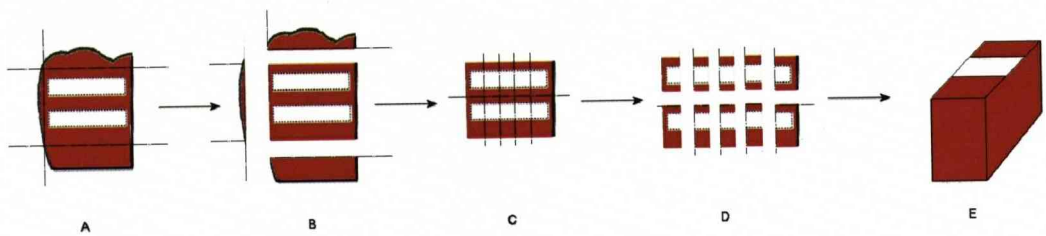


Figure 4.3 Diagrammatic representation of further sectioning to illustrate harvesting of enamel samples prior to erosion

All slabs from each tooth were mounted on glass rods using thermoplastic impression material (Green Stick Impression Compound, Kerr, Sybron, USA) so that the enamel from the buccal surface was facing outwards (Figure 4.4).

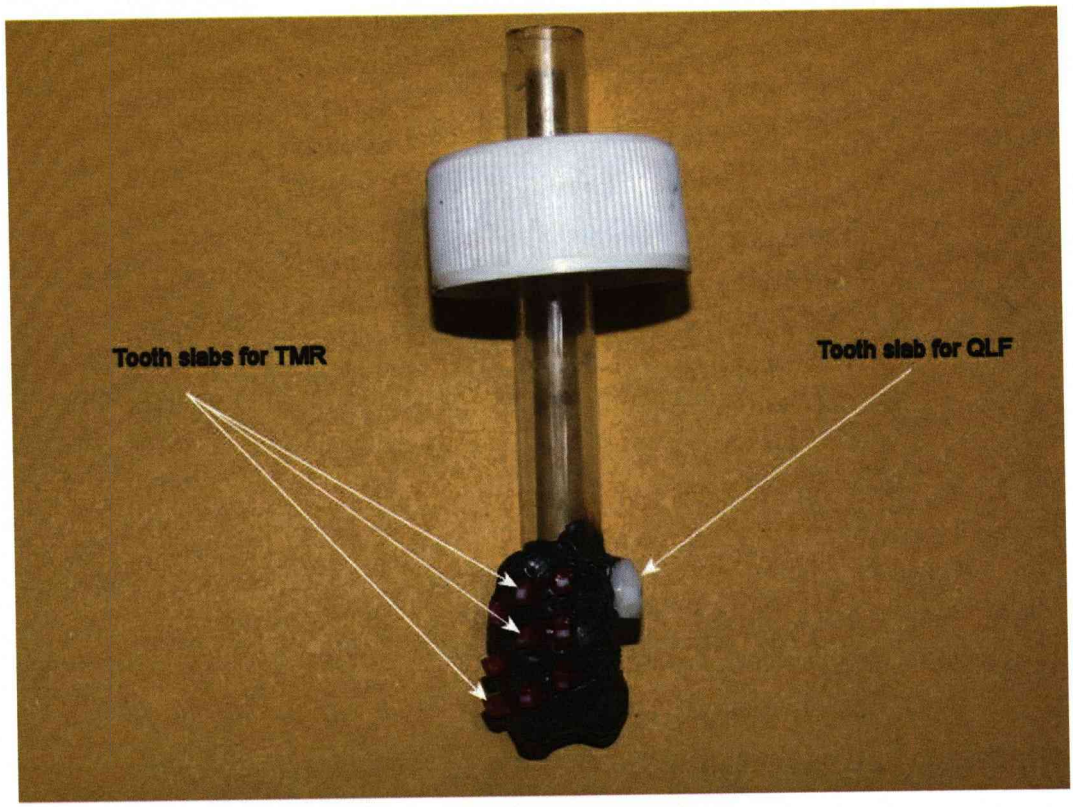


Figure 4.4 Tooth slabs mounted on a glass rod ready for immersion in orange juice

4.3.3 Exposure to erosive conditions

Subsequently the bovine and human enamel samples were treated in the same way but in separate experiments.

All specimens mounted on glass rods were placed in plastic 30 ml beakers and were subject to artificial erosion by immersing them in 20 ml of agitated orange juice (Tesco Value, Tesco, UK, batch no X1124) at room temperature. Every 30 minutes the glass rods were removed from the experimental containers, rinsed with double distilled water then dried for 20 minutes, the QLF images of the relevant slabs were taken and one smaller slab was detached for further TMR analysis. After that the glass rods were placed back into the plastic beakers with the same acidic agent for further erosive challenge. The experiment was carried out for up to 300 minutes total exposure to orange juice.

4.3.4 Analysis of the samples

Progression of dental erosion was quantified in the corresponding portion of each tooth using QLF and TMR techniques. QLF images were analysed with QLF™ software (version 2.00c, Inspektor Research Systems BV, the Netherlands) and fluorescence loss (ΔQ) of both bovine and human enamel quantified (Figure 4.5). Those enamel slabs subjected to TMR analysis were processed as described in Chapter 2. Slabs of bovine teeth were cut into 3 or 4 sections, while the human ones into 1 or 2 sections due to the limitation of human teeth sizes. The microradiographs were analysed with TMRW software (version 1.22, Inspektor Research Systems BV, the Netherlands) and data for total mineral loss (ΔZ) and lesion depth (ld) was obtained by a two-step image analysis technique described by Amaechi *et al* (1998a). To assess the depth of crater (ldc) created by erosion the TMR images were reanalysed calculating the mineral loss (ΔZs) and lesion depth (lds) of only the softened surface (Figure 4.6). The depth of crater was computed by subtracting the lesion depth of only the softened surface from total lesion depth (ldc = ld - lds).

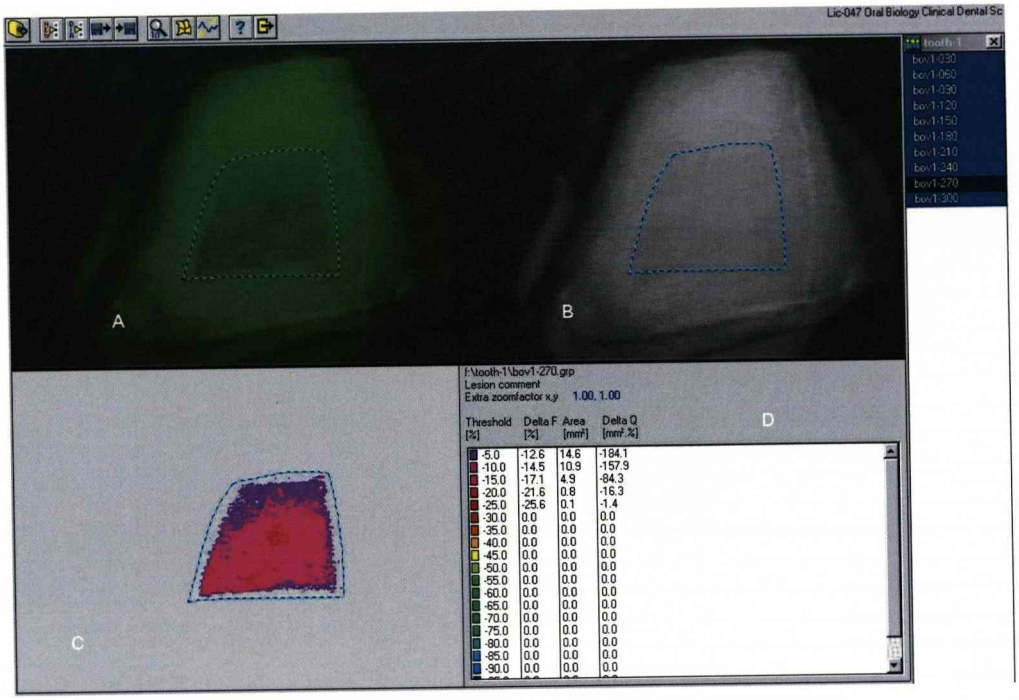
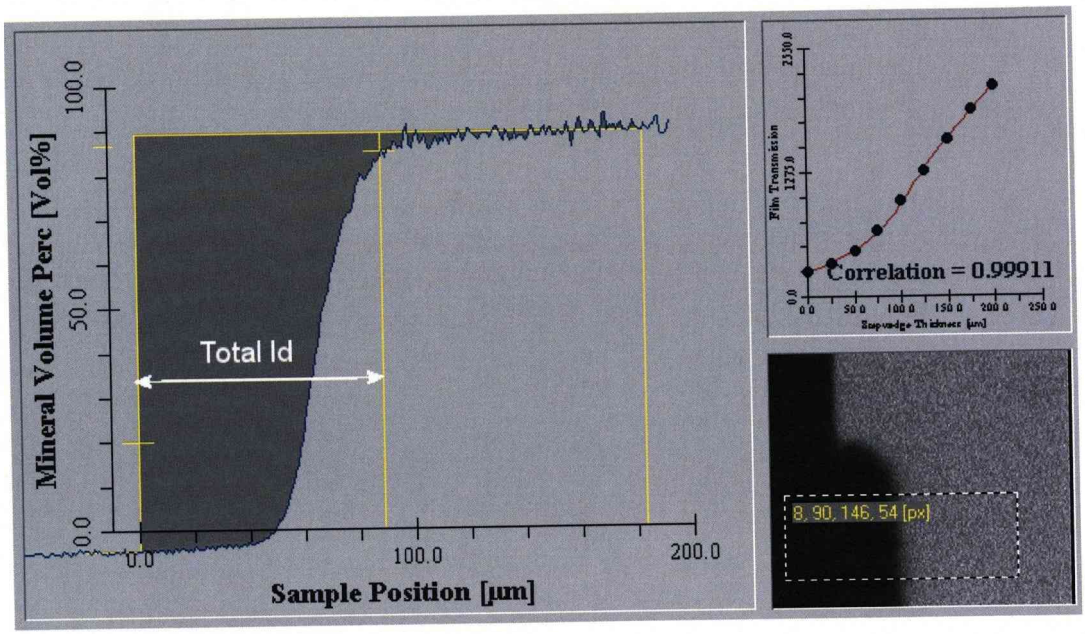
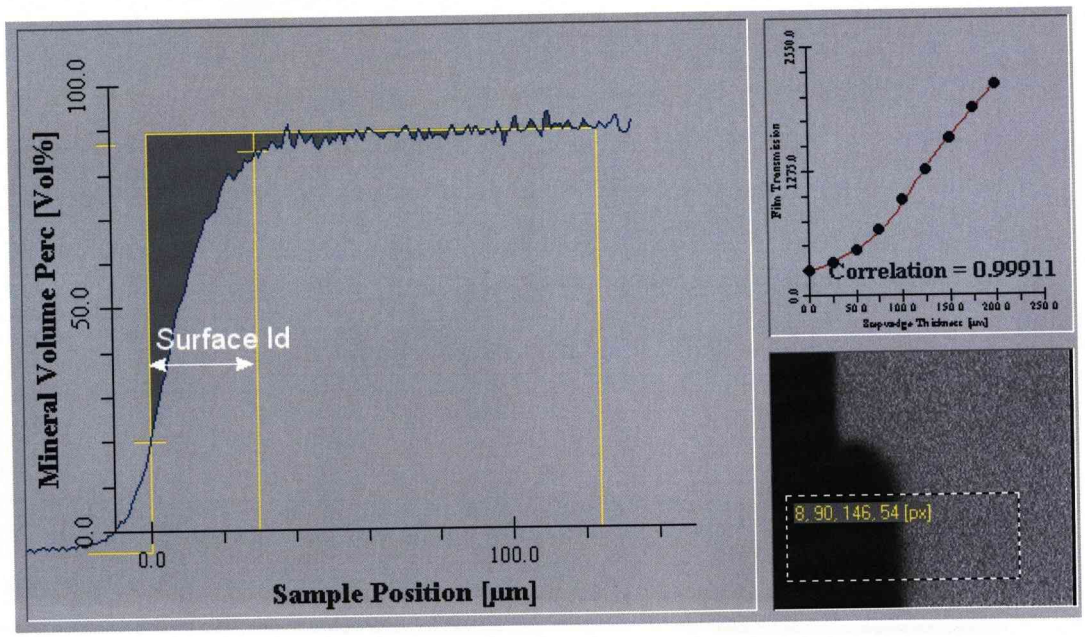


Figure 4.5 QLF image of bovine tooth subjected to 270 minutes of erosive challenge analyzed by QLF™ software,version 2.00c package. A – original image, B – reconstructed black and white image, C – area of lesion included in reference patch, D – calculated loss of fluorescence and area at different thresholds



A



B

Figure 4.6 TMR analysis of crater depth. A – total lesion depth. B – lesion depth of only the softened surface. The depth of crater was calculated as total lesion depth minus lesion depth of softened surface

4.3.5 Statistical analysis

The number of sections for each tooth slab processed for TMR analysis was between 1 and 4 and the mean values were calculated to get the final reading for each particular tooth.

The data were analysed using simple regression analysis, where Pearson correlation coefficient, squared Pearson and adjusted squared Pearson correlation coefficient were calculated and regression equations were obtained using MINITAB® Release 14.20 software.

4.4 Results

The mean pH of orange juice used in both experiments was 3.55 ± 0.01 for bovine enamel and 3.63 ± 0.01 for human enamel.

4.4.1 Bovine enamel

The data representing the changes in bovine enamel (Figure 4.7) measured by both techniques employed in this experiment are shown in Tables 4.1 – 4.6. TMR and QLF demonstrated strong linear correlation between mineral loss of enamel during constant erosive challenge with orange juice and time of exposure to it. While TMR illustrated the direct measurement of changes in mineral content, the QLF – through changes in tooth fluorescence, which indirectly corresponds to mineral changes of dissolving enamel. Adjusted squared Pearson correlation coefficient (adj.R^2) for total mineral loss was 99.3%, for total lesion depth – 99.1% and for loss of fluorescence (ΔQ) – 98.2% (Figures 4.8 – 4.10).

There was observed a strong correlation between TMR and QLF monitoring the progression of dental erosion within time, adj.R^2 = 98.4 and 98.8 for total mineral loss and lesion depth respectively (Figures 4.11 and 4.12).

By plotting the average values of mineral loss of only the surface of lesion, excluding the depth of crater, against time of erosive challenge, as well as the depth of crater alone against time, the strong linear correlation was detected with adjusted squared Pearson correlation coefficients obtained as 76.3% and 99.5% respectively (Figures 4.13 and 4.14). While matching these TMR values against QLF data obtained for the same teeth the adj.R^2 was 78.9% and 98.4% respectively (Figures 4.15 and 4.16).

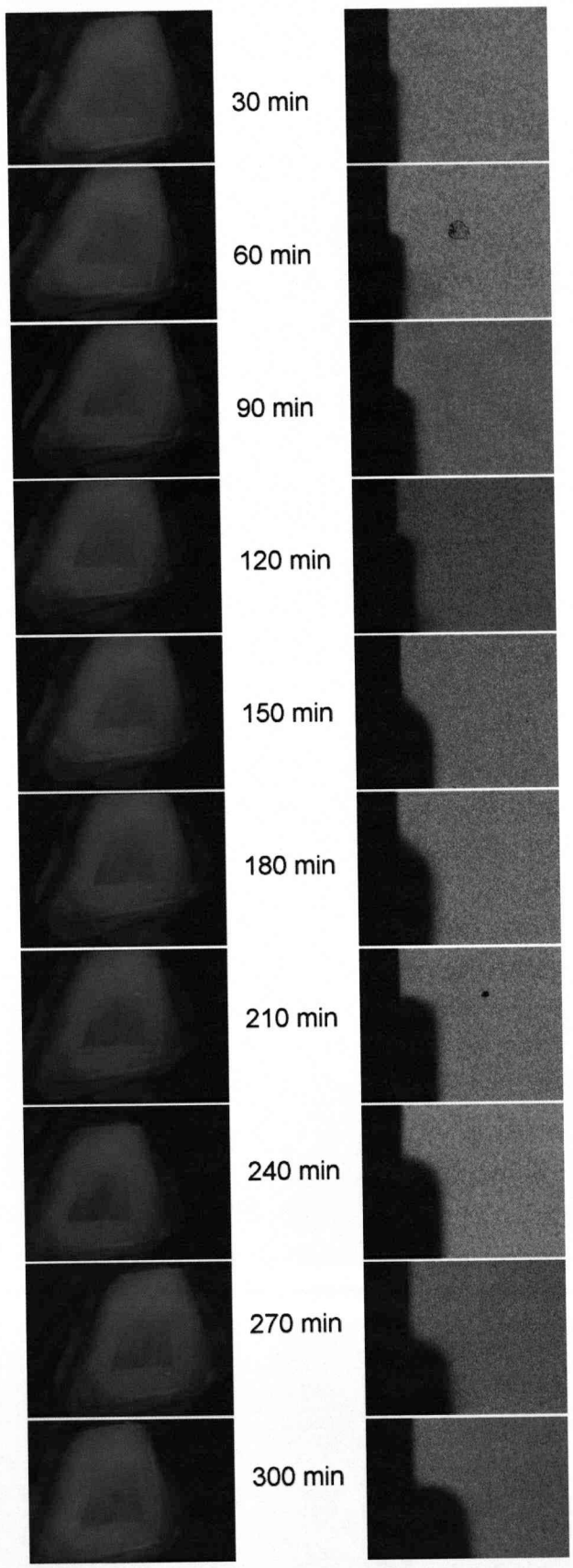


Figure 4.7 QLF and corresponding TMR images of a bovine tooth subjected to 30 - 300 minutes of agitated orange juice

Table 4.1 Total mineral loss of eroded lesions as measured by TMR (ΔZ, vol%·μm)

Tooth	Time of exposure, minutes									
number	30	60	90	120	150	180	210	240	270	300
1	718.9	1253.6	1894.6	2019.1	2625.1	3529.2	4317.9	4873.4	5810.0	6720.7
2	744.9	1048.1	1727.9	1731.6	3372.9	4438.5	3532.8	3706.0	4813.6	5774.8
3	668.9	1023.5	1165.8	1772.6	2147.3	2519.9	2795.4	3131.1	3664.7	3799.1
4	726.7	1049.9	1309.4	2553.1	2587.9	4026.6	3936.5	4605.5	5027.0	5689.7
5	562.8	1516.1	1615.3	1762.0	3206.6	3492.5	3925.2	5058.4	5156.3	5842.2
6	993.4	1299.5	1990.5	2777.0	3681.1	4247.6	4780.8	4713.6	5422.7	5946.8
7	757.0	1448.1	2440.0	2741.9	4342.4	4684.0	4873.9	6042.0	6306.8	6237.5
8	832.3	1403.7	2242.7	3009.5	3970.7	4614.0	6037.6	6058.9	6431.9	7578.1
9	1100.2	1928.6	2912.9	3215.5	3847.0	3854.7	5494.4	5589.8	5703.4	6467.3
10	953.6	1819.2	2562.2	3122.5	4481.1	5270.4	6327.5	6467.4	7096.1	6939.8
11	814.3	1293.3	1813.4	2084.8	3480.7	3637.6	4091.6	5138.6	5967.4	6012.8
12	737.1	1795.4	2193.4	2659.6	4105.8	4620.3	4786.5	5337.3	6161.1	6384.9
13	1021.2	1275.4	2164.8	3149.0	3838.7	4534.1	4984.1	5252.7	5388.8	6254.0
Mean	**817.8**	**1396.5**	**2002.5**	**2507.6**	**3514.4**	**4113.0**	**4606.5**	**5075.0**	**5611.5**	**6126.7**
SD	**155.8**	**299.1**	**492.2**	**563.5**	**710.4**	**709.4**	**990.3**	**924.5**	**861.1**	**875.1**

Table 4.2 Total depth of eroded lesions as measured by TMR (μm)

	Time of exposure, minutes									
Tooth number	**30**	**60**	**90**	**120**	**150**	**180**	**210**	**240**	**270**	**300**
1	21.7	23.0	32.8	43.8	47.1	55.4	64.0	68.3	82.6	92.3
2	18.9	25.8	37.3	35.4	53.4	66.3	54.5	60.2	71.3	80.2
3	20.9	27.5	26.1	37.5	48.4	44.4	46.3	58.0	63.7	61.0
4	19.2	25.2	29.1	47.2	46.6	62.2	66.2	69.8	78.5	84.4
5	17.6	33.7	38.8	36.0	53.3	65.7	73.4	86.3	76.9	90.1
6	27.0	28.5	42.1	50.1	59.2	62.7	86.0	70.0	81.2	89.5
7	21.5	28.9	41.9	45.1	61.1	68.3	72.0	80.7	84.7	84.3
8	22.6	29.2	41.2	47.9	66.1	68.6	83.3	83.4	84.7	99.7
9	24.3	30.8	42.1	49.3	56.6	58.4	76.2	80.5	83.4	89.9
10	19.7	29.7	37.0	51.5	63.8	72.1	85.4	94.9	96.1	92.8
11	18.6	25.1	30.8	35.9	54.1	54.6	59.9	71.3	79.9	83.7
12	19.5	31.7	40.8	43.0	59.3	64.5	70.7	80.2	88.4	82.4
13	20.9	23.5	32.9	46.6	54.4	61.5	64.0	73.6	70.0	80.5
Mean	**21.0**	**27.5**	**35.4**	**42.2**	**52.7**	**60.7**	**66.1**	**70.5**	**77.0**	**83.1**
SD	**2.6**	**3.3**	**5.5**	**5.8**	**6.2**	**7.3**	**11.9**	**10.4**	**8.4**	**9.2**

Table 4.3 Mineral loss of eroded surface (without crater) as measured by TMR (ΔZ, vol%·μm)

	Time of exposure, minutes									
Tooth number	**30**	**60**	**90**	**120**	**150**	**180**	**210**	**240**	**270**	**300**
1	349.9	254.1	349.8	734.5	901.5	955.6	565.8	601.5	618.5	698.9
2	310.7	477.3	621.4	585.9	554.0	638.6	529.7	1449.8	568.2	518.9
3	482.5	422.9	392.7	570.4	830.3	593.6	493.0	698.3	720.6	598.0
4	452.2	458.9	433.7	604.5	559.1	610.9	771.0	715.3	730.7	698.3
5	292.7	555.5	726.9	549.1	648.1	946.8	887.2	838.6	691.7	993.6
6	501.9	504.5	829.9	577.4	655.7	731.2	897.6	579.7	693.0	867.7
7	443.4	462.3	479.5	479.9	424.1	543.5	594.3	463.0	474.5	518.6
8	421.6	414.8	427.0	302.9	644.3	760.6	703.3	574.6	466.6	681.5
9	400.0	355.6	402.9	530.9	478.1	567.4	606.5	748.3	707.0	634.8
10	231.4	231.1	290.6	473.9	636.5	451.6	738.6	954.1	494.7	527.5
11	238.2	315.8	318.3	375.4	457.9	463.7	465.3	480.1	401.6	707.7
12	261.1	324.3	485.2	460.2	450.0	412.2	528.7	632.3	777.4	465.9
13	350.5	283.5	275.8	341.5	387.7	395.6	360.5	459.7	379.6	368.0
Mean	**364.3**	**389.3**	**464.1**	**506.6**	**586.7**	**620.9**	**626.3**	**707.3**	**594.2**	**636.9**
SD	**93.1**	**102.4**	**168.2**	**118.7**	**154.8**	**184.3**	**163.2**	**266.9**	**137.0**	**167.9**

Table 4.4 Lesion depth of eroded surface (without crater) as measured by TMR (μm)

	Time of exposure, minutes									
Tooth number	**30**	**60**	**90**	**120**	**150**	**180**	**210**	**240**	**270**	**300**
1	17.2	11.3	14.9	28.6	27.0	25.6	21.9	20.8	24.4	24.1
2	13.1	18.7	23.7	21.7	21.4	23.8	19.4	33.5	23.1	19.1
3	18.4	20.2	16.9	23.5	32.4	22.4	19.8	29.3	29.3	23.3
4	15.8	17.9	18.8	25.1	23.4	23.8	30.3	25.7	27.9	25.1
5	14.2	22.3	27.9	21.7	24.0	36.9	38.6	35.2	24.2	35.1
6	20.8	18.8	28.4	24.1	24.8	23.9	43.1	22.7	27.3	31.9
7	17.4	17.0	19.5	19.0	17.3	21.7	23.9	16.9	17.8	18.2
8	17.0	16.9	19.7	11.9	27.5	24.9	24.3	21.6	17.3	23.2
9	15.8	12.8	14.0	18.5	17.8	20.8	20.9	24.2	25.4	22.3
10	11.2	11.3	11.6	21.2	21.3	17.3	23.4	30.1	15.1	19.2
11	11.8	13.7	13.4	15.8	19.9	18.8	18.8	19.0	16.9	23.5
12	13.9	14.6	21.2	18.1	18.4	16.8	22.3	25.8	25.9	15.2
13	12.9	12.0	11.6	14.9	15.5	14.9	12.8	17.4	12.5	12.7
Mean	**15.3**	**16.0**	**18.6**	**20.3**	**22.4**	**22.4**	**24.6**	**24.8**	**22.1**	**22.5**
SD	**2.8**	**3.6**	**5.6**	**4.6**	**4.8**	**5.5**	**8.3**	**5.9**	**5.5**	**6.1**

Table 4.5 Depth of crater of eroded lesions as measured by TMR (μm)

	Time of exposure, minutes									
Tooth number	**30**	**60**	**90**	**120**	**150**	**180**	**210**	**240**	**270**	**300**
1	4.5	11.7	17.8	15.3	20.1	29.9	42.1	47.6	58.2	68.2
2	5.9	7.1	13.7	13.7	32.0	42.5	35.1	26.7	48.2	61.0
3	2.5	7.3	9.1	14.0	16.0	22.0	26.6	28.7	34.4	37.8
4	3.3	7.3	10.4	22.2	23.2	38.4	35.9	44.1	50.5	59.4
5	3.4	11.4	10.9	14.3	29.3	28.7	34.7	51.2	52.7	55.0
6	6.2	9.7	13.7	26.0	34.5	38.9	42.9	47.3	54.0	57.6
7	4.1	11.9	22.4	26.0	43.7	46.7	48.1	63.7	66.9	66.1
8	5.6	12.3	21.5	36.0	38.6	43.7	59.1	61.9	67.4	76.5
9	8.4	18.0	28.1	30.8	38.8	37.6	55.2	36.2	58.0	67.5
10	8.5	18.4	25.4	30.3	42.5	54.8	62.0	64.8	81.0	73.7
11	6.9	11.4	17.4	20.1	34.2	35.9	41.1	52.3	63.0	60.2
12	5.6	17.1	19.7	24.8	42.0	48.0	48.4	54.4	62.5	67.2
13	8.0	11.5	21.3	31.7	38.9	46.6	51.2	56.3	57.5	67.8
Mean	**5.6**	**11.9**	**17.8**	**23.5**	**33.4**	**39.5**	**44.8**	**48.9**	**58.0**	**62.9**
SD	**2.0**	**3.8**	**5.9**	**7.6**	**8.9**	**9.0**	**10.4**	**12.4**	**11.2**	**9.8**

Table 4.6 Loss of fluorescence of eroded lesions as measured by QLF (ΔQ, $mm^2 \cdot \%$)

	Time of exposure, minutes									
Tooth number	**30**	**60**	**90**	**120**	**150**	**180**	**210**	**240**	**270**	**300**
1	1.1	1.8	3.9	9.5	11.4	29.7	37.2	40.3	43.8	45.5
2	5.1	6.8	9.8	12.6	16.8	24.3	18.6	19.1	33.7	35.0
3	1.3	1.9	6.3	8.4	17.9	18.4	21.0	20.8	43.9	62.1
4	1.4	2.5	6.3	7.3	18.8	19.7	21.8	26.8	32.3	34.3
5	1.4	4.3	8.4	10.2	11.3	26.7	19.5	28.7	29.7	32.7
6	0.4	4.6	8.5	10.7	12.0	13.8	16.5	17.6	20.3	26.8
7	2.6	8.8	19.3	31.1	39.4	43.8	57.8	64.4	75.0	76.3
8	17.0	35.2	42.3	63.5	70.7	80.0	99.1	86.5	93.8	94.5
9	7.9	39.9	50.9	79.3	75.3	94.8	96.7	96.5	115.1	115.5
10	7.5	19.0	24.1	26.2	34.4	33.3	44.5	38.9	55.8	54.4
11	4.7	16.6	23.7	43.7	51.3	53.9	74.5	75.0	79.1	87.7
12	16.8	25.3	65.9	79.9	89.3	93.3	96.6	102.9	115.9	116.9
13	16.9	26.2	34.2	40.9	43.7	39.5	58.7	60.5	71.4	61.5
Mean	**6.5**	**14.8**	**23.4**	**32.6**	**37.9**	**43.9**	**51.0**	**52.2**	**62.3**	**64.9**
SD	**6.4**	**13.3**	**19.7**	**27.0**	**26.8**	**28.3**	**32.1**	**30.6**	**32.2**	**31.1**

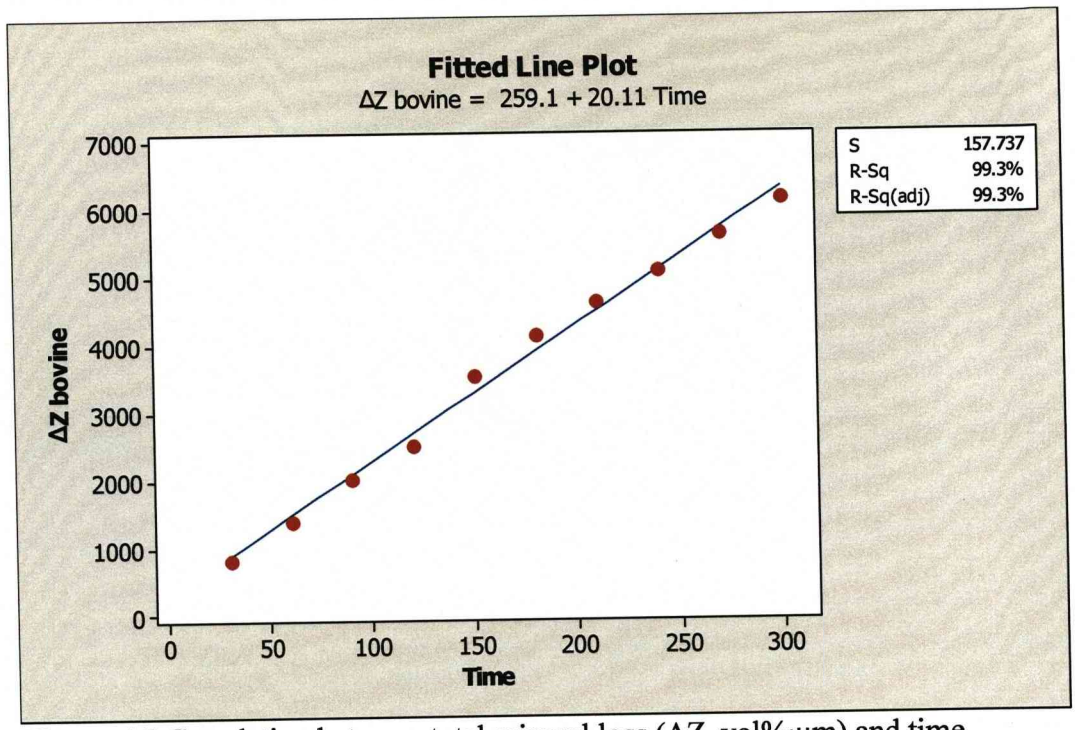


Figure 4.8 Correlation between total mineral loss (ΔZ, vol%·μm) and time (minutes) of erosive exposure in bovine enamel.

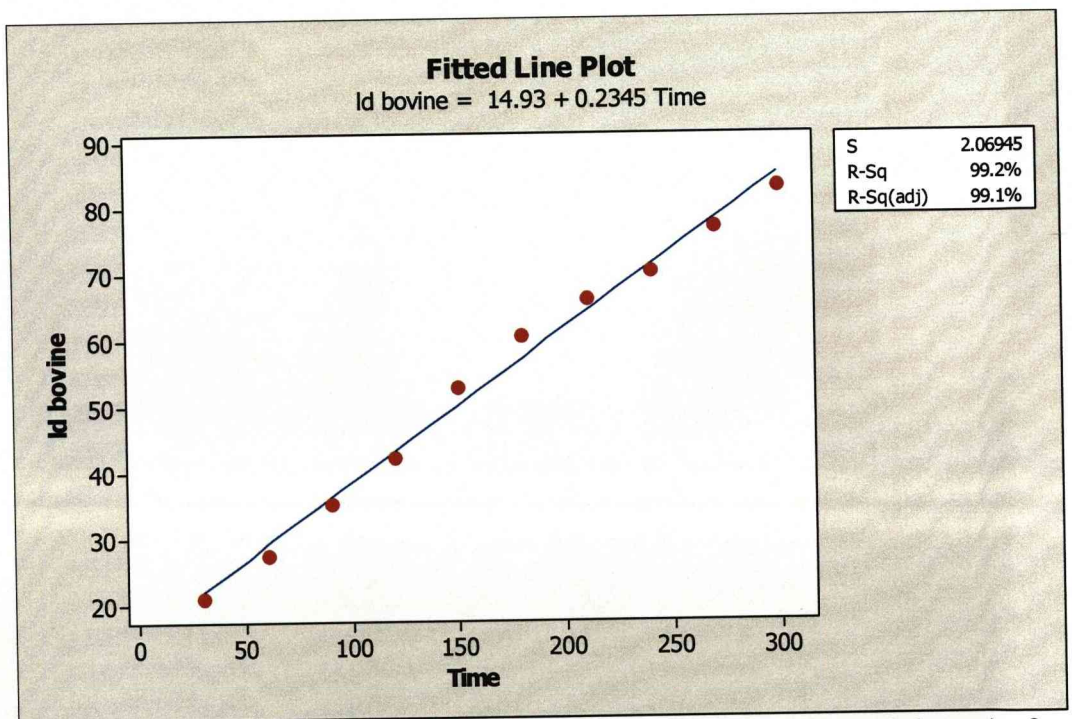


Figure 4.9 Correlation between total lesion depth (ld, μm) and time (minutes) of erosive exposure in bovine enamel

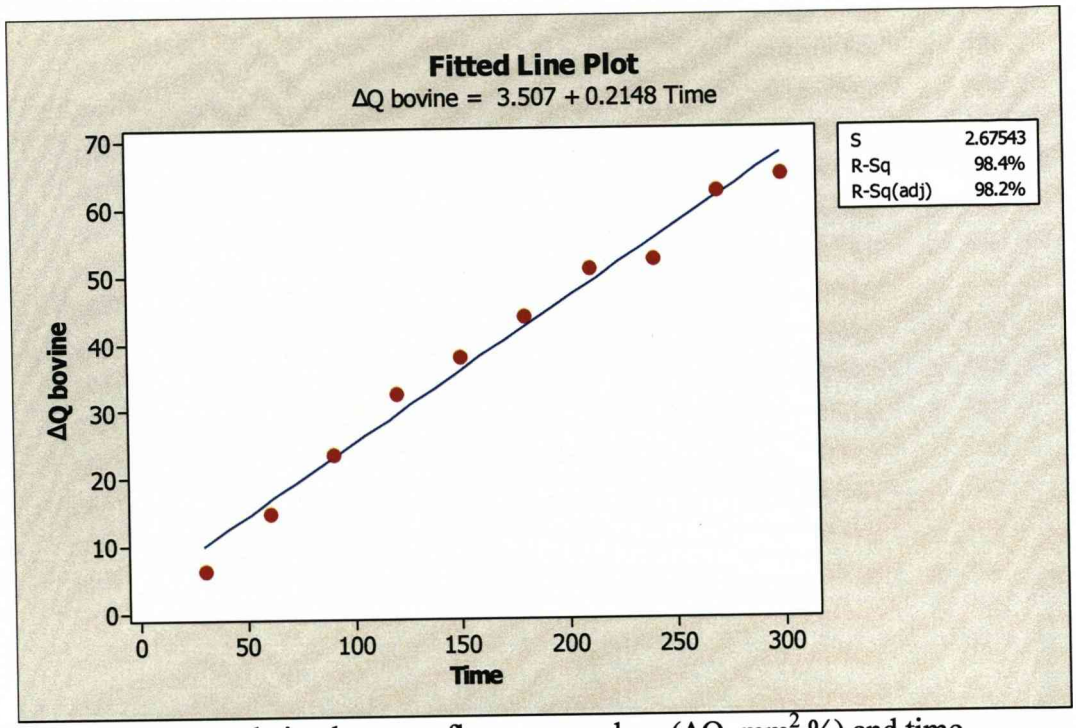


Figure 4.10 Correlation between fluorescence loss (ΔQ, $mm^2 \cdot \%$) and time (minutes) of erosive exposure in bovine enamel.

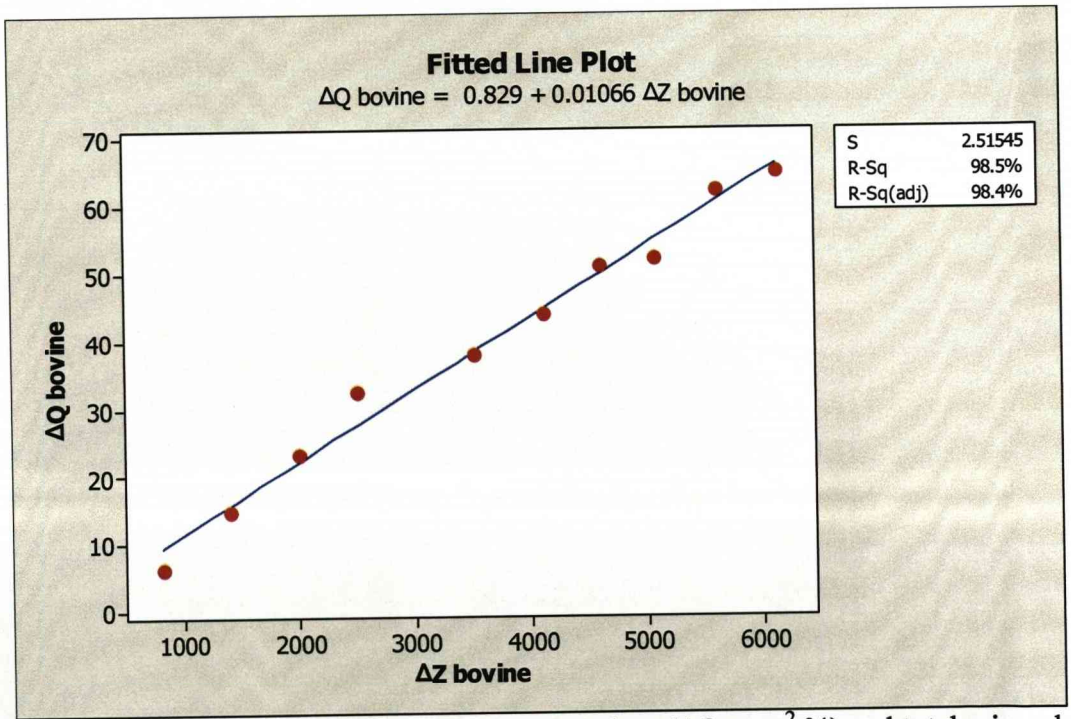


Figure 4.11 Correlation between fluorescence loss (ΔQ, $mm^2 \cdot \%$) and total mineral loss (ΔZ, vol%·μm) of erosion in bovine enamel.

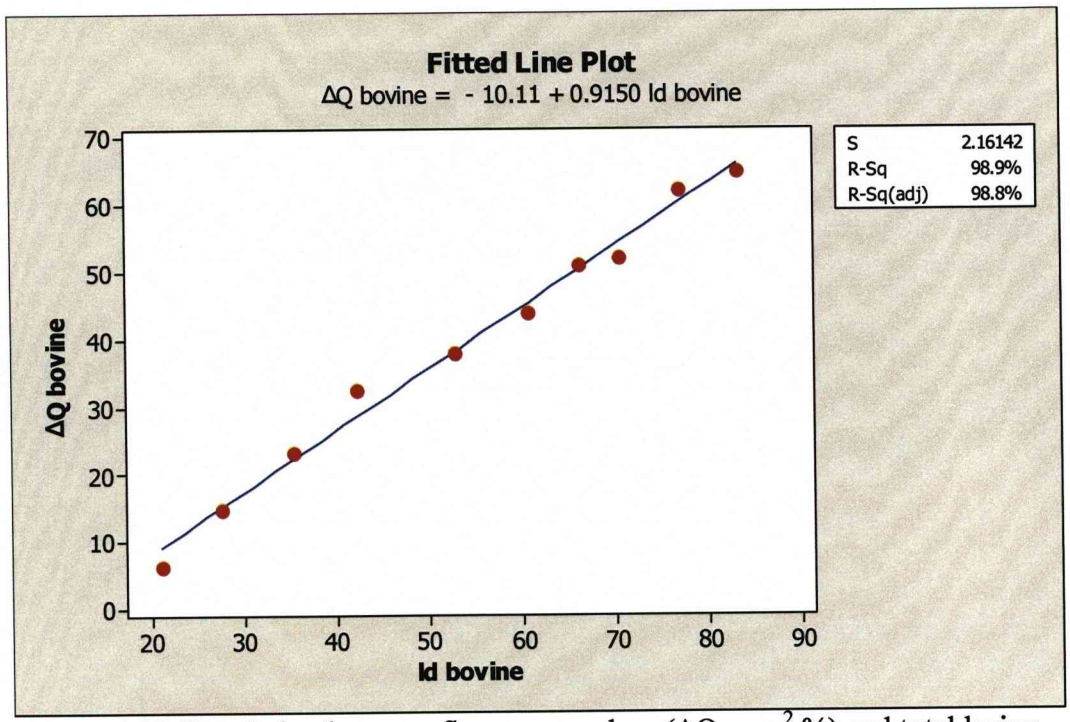


Figure 4.12 Correlation between fluorescence loss (ΔQ, $mm^2 \cdot \%$) and total lesion depth (ld, μm) of erosion in bovine enamel

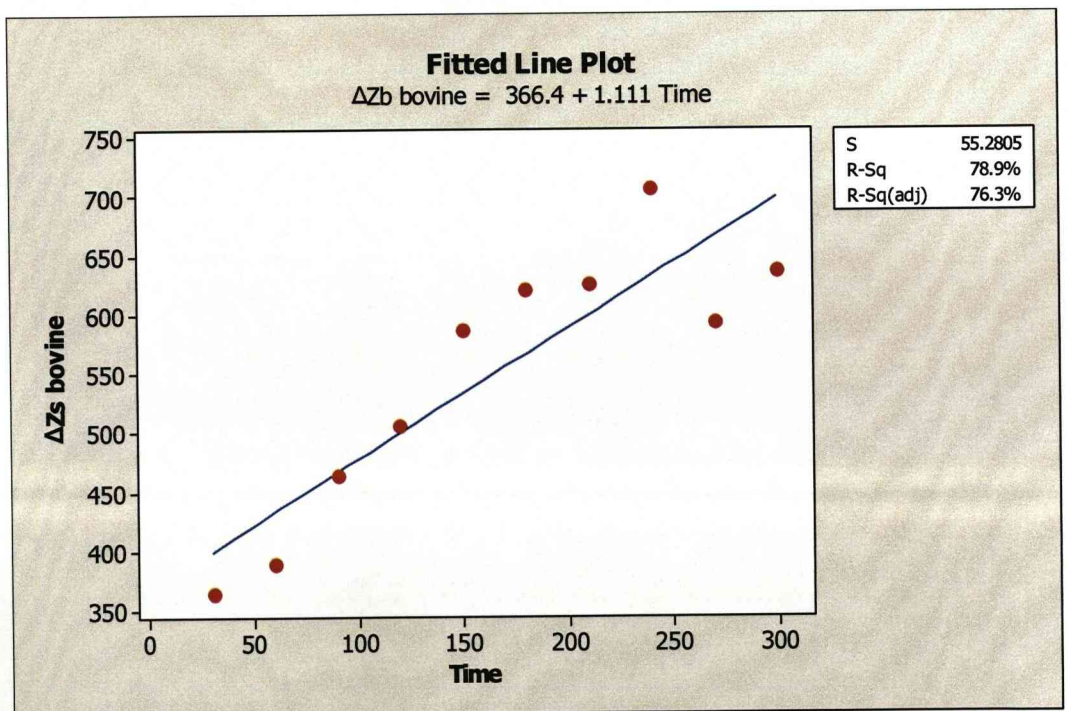


Figure 4.13 Correlation between only surface mineral loss (ΔZs, vol%·μm) and time (minutes) of erosive exposure in bovine enamel

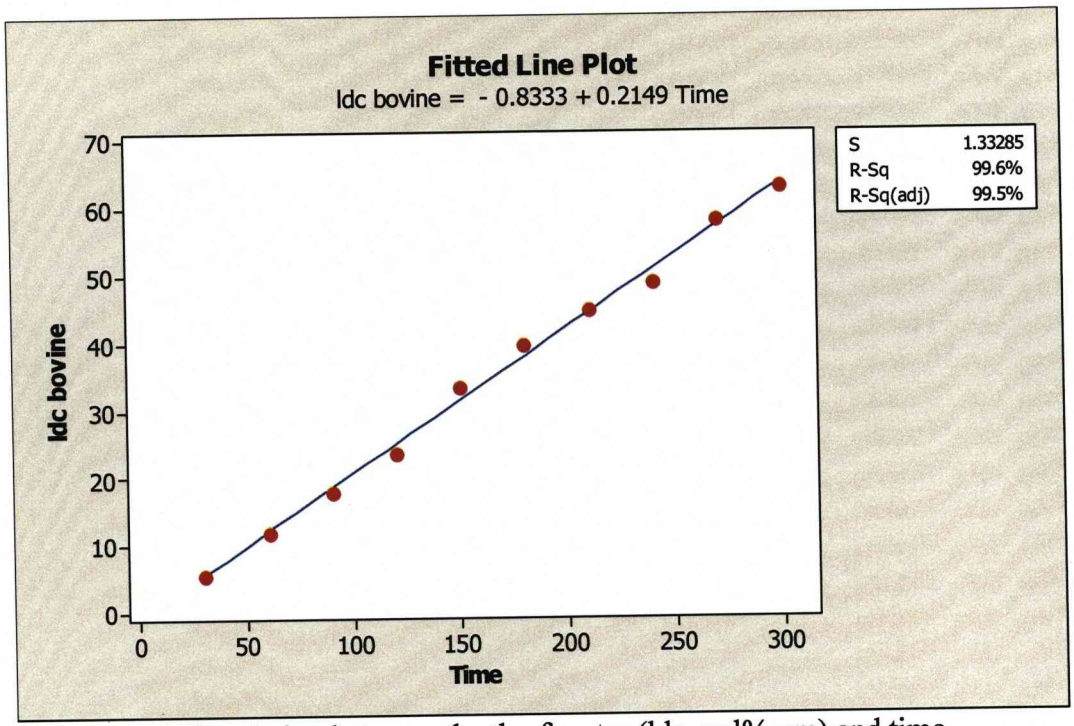


Figure 4.14 Correlation between depth of crater (ldc, vol%·µm) and time (minutes) of erosive exposure in bovine enamel

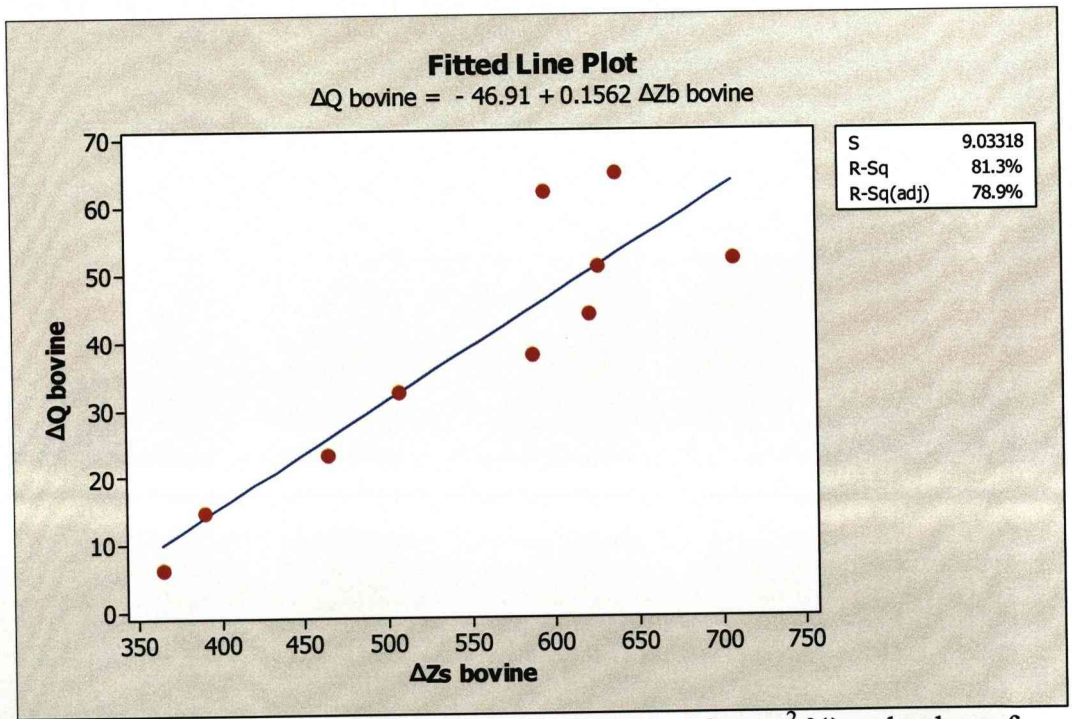


Figure 4.15 Correlation between fluorescence loss (ΔQ, mm^2·%) and only surface mineral loss (ΔZs, vol%·µm) of erosion in bovine enamel

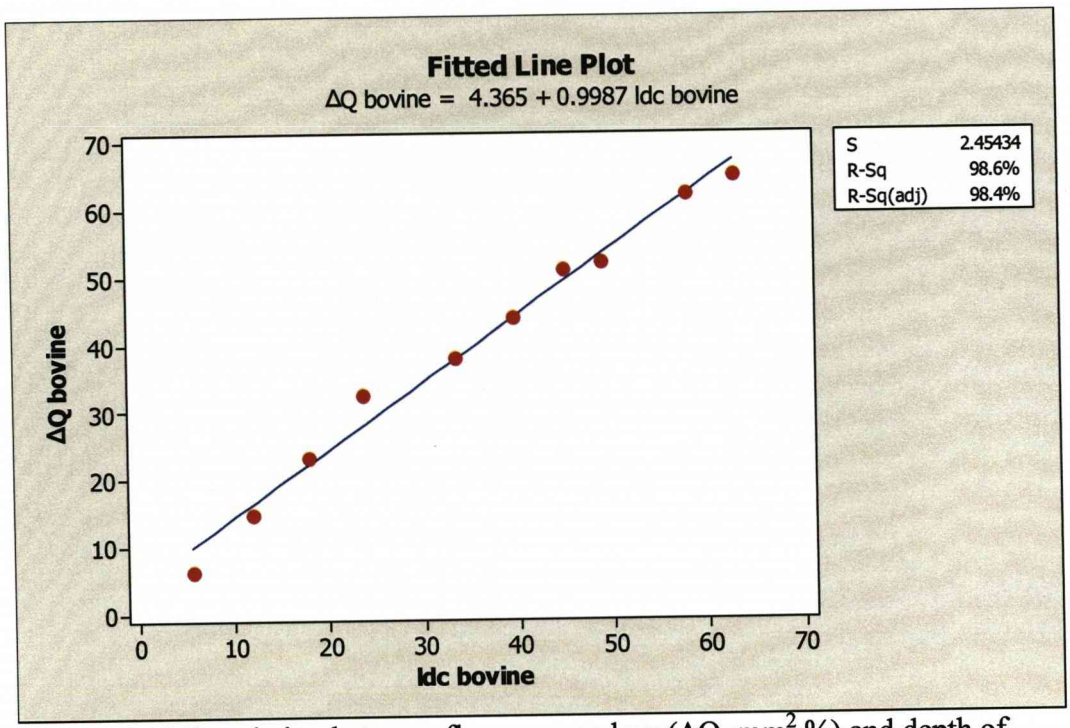


Figure 4.16 Correlation between fluorescence loss (ΔQ, $mm^2 \cdot \%$) and depth of crater of erosion(ldc, μm) in bovine enamel

4.4.2 Human enamel

Tables 4.7 – 4.12 show longitudinal statistical data of changes in mineral content and fluorescence of human enamel after continuous exposure to test samples of orange juice for up to 300 minutes. For some enamel sections the data are missing due to loss of samples during the rather damaging process of preparing sections for TMR analysis. The size of buccal surface of human molars in comparison with bovine incisors put a limit to the width of slabs for the study with such design, which necessitated having only one or two sections, bearing a lesion, from each slab.

A strong linear correlation was observed between time of exposure to the erosive challenge and the degree of loss of enamel minerals measured by total mineral loss (ΔZ), lesion depth (ld) and fluorescence (ΔQ) with squared Pearson correlation coefficient (adj.R^2) equal to 99.6%, 92.4% and 99.4% respectively (Figures 4.17 – 4.19).

Again as with bovine teeth a strong correlation for human teeth was observed between TMR and QLF to monitor changes in the progression of dental erosion within time factor with adj.R^2 = 99.3 and 91.1 for total mineral loss and total lesion depth respectively (Figures 4.20 and 4.21).

While comparing the TMR data plotting against time for mineral loss for only softened surface of eroded enamel with the depth of crater of erosion, which

does not exclude the crater of lesion, adjusted squared Pearson correlation coefficient was recorded as 76.3% and 96.0% respectively (Figures 4.22 – 4.23). The similar relationship was observed when the loss of fluorescence (ΔQ) was drawn against the only surface mineral loss (ΔZs) and crater depth (ldc) with adj.R^2 equals 76.0% and 95.4% respectively (Figures 4.24 – 4.25).

Table 4.7 Total mineral loss of eroded lesions as measured by TMR (ΔZ, vol%·μm)

	Time of exposure, minutes								
Tooth number	**60**	**90**	**120**	**150**	**180**	**210**	**240**	**270**	**300**
1	643.7	727.6	611.0	1026.6	-	2097.6	2292.2	-	2129.4
2	-	-	728.7	1158.8	1550.0	2160.6	-	-	2640.1
3	-	691.8	-	1215.8	1137.5	1220.3	-	-	-
4	962.6	-	-	1311.1	1344.3	1622.3	-	1911.4	2223.3
5	415.1	-	-	1188.1	1228.1	1290.2	-	1432.3	2045.8
6	-	1004.9	-	1255.3	1774.7	2004.7	2101.3	-	2372.2
7	411.6	-	912.4	1164.4	1269.8	1467.5	1652.1	1996.3	1883.3
8	545.2	-	899.1	1093.8	1233.7	1289.6	1716.6	2714.7	2366.9
9	417.6	750.0	1151.3	1342.7	1567.4	-	1714.6	2012.9	2184.7
10	600.7	736.1	935.7	1192.4	1155.1	1311.1	1304.5	1446.8	2064.6
11	571.6	694.6	822.9	1169.9	1049.8	1294.7	1613.1	-	2405.1
12	581.8	628.3	1061.1	895.6	1487.2	1799.8	1911.7	-	2298.1
13	498.4	703.7	926.0	1203.2	1511.3	1930.7	1905.4	2207.8	2276.4
n	*10*	*8*	*9*	*13*	*12*	*11*	*9*	*7*	*11*
Mean	**564.8**	**742.1**	**894.2**	**1170.6**	**1359.1**	**1624.1**	**1801.3**	**1960.3**	**2240.8**
SD	**162.8**	**112.5**	**161.9**	**116.5**	**217.3**	**356.7**	**290.1**	**443.3**	**199.3**

Table 4.8 Total depth of eroded lesions as measured by TMR (μm)

	Time of exposure, minutes								
Tooth number	**60**	**90**	**120**	**150**	**180**	**210**	**240**	**270**	**300**
1	14.9	17.1	13.5	24.2	-	36.8	36.4	-	39.4
2	-	-	17.9	20.2	26.5	41.9	-	-	38.7
3	-	14.4	-	21.4	26.3	26.1	-	-	-
4	21.5	-	-	27.0	24.4	26.9	-	27.0	39.8
5	12.9	-	-	25.9	26.3	24.4	-	27.8	35.8
6	-	27.5	-	30.6	36.1	32.9	34.9	-	40.5
7	16.3	-	19.6	26.3	28.8	27.3	29.1	39.8	33.0
8	19.9	-	19.9	22.8	22.2	24.1	28.9	63.5	37.3
9	20.3	16.7	26.7	26.7	29.1	-	27.0	35.5	39.5
10	13.8	15.8	19.4	25.2	24.6	25.4	28.2	30.1	50.6
11	15.6	18.2	17.7	24.1	28.5	24.0	32.0	-	36.4
12	13.0	14.3	23.7	19.4	26.9	29.3	31.8	-	35.8
13	12.3	18.1	17.7	23.8	27.1	32.2	32.0	37.2	36.4
n	*10*	*8*	*9*	*13*	*12*	*11*	*9*	*7*	*11*
Mean	**16.0**	**17.7**	**19.5**	**24.4**	**27.2**	**29.3**	**31.1**	**37.3**	**38.6**
SD	**3.4**	**4.2**	**3.8**	**3.1**	**3.4**	**5.6**	**3.1**	**12.6**	**4.4**

Table 4.9 Mineral loss of eroded surface (without crater) as measured by TMR (ΔZ, vol%·μm)

Tooth number	Time of exposure, minutes								
	60	**90**	**120**	**150**	**180**	**210**	**240**	**270**	**300**
1	223.3	197.5	189.7	411.1	-	407.6	362.2	-	580.6
2	-	-	279.4	195.9	381.6	599.7	-	-	330.8
3	-	261.7	-	172.7	471.8	378.3	-	-	-
4	297.2	-	-	523.9	377.0	302.8	-	194.9	445.7
5	299.8	-	-	564.5	476.4	325.7	-	471.7	491.9
6	-	562.5	-	531.6	539.9	498.8	470.9	-	448.9
7	336.6	-	320.4	469.6	534.5	304.0	393.2	558.3	384.0
8	454.2	-	286.4	401.0	307.8	310.5	298.5	769.9	400.0
9	215.5	203.3	423.8	317.3	436.0	-	287.4	555.3	560.4
10	224.4	245.9	355.4	462.0	377.0	253.1	677.5	530.6	710.0
11	255.4	300.1	179.7	325.9	451.9	419.2	483.3	-	353.2
12	179.9	192.1	369.5	373.9	345.6	270.8	312.0	-	333.8
13	228.9	324.0	301.0	327.4	426.8	335.8	298.1	322.5	276.0
n	*10*	*8*	*9*	*13*	*12*	*11*	*9*	*7*	*11*
Mean	**271.5**	**285.9**	**300.6**	**390.5**	**427.2**	**367.2**	**398.1**	**486.2**	**442.9**
SD	**79.7**	**121.8**	**79.8**	**122.1**	**72.2**	**101.0**	**128.2**	**184.5**	**125.1**

Table 4.10 Lesion depth of eroded surface (without crater) as measured by TMR (μm)

	Time of exposure, minutes								
Tooth number	**60**	**90**	**120**	**150**	**180**	**210**	**240**	**270**	**300**
1	9.9	10.8	8.5	16.8	-	17.5	14.4	-	21.0
2	-	-	12.3	9.2	13.0	23.6	-	-	12.7
3	-	9.4	-	9.3	18.4	16.4	-	-	-
4	13.7	-	-	17.2	13.3	11.7	-	7.7	19.1
5	11.3	-	-	18.1	17.3	13.6	-	16.6	17.4
6	-	21.4	-	21.5	21.6	15.8	16.4	-	18.5
7	15.3	-	12.5	17.7	20.0	13.3	14.3	23.1	15.5
8	18.7	-	12.6	14.2	11.4	12.8	12.0	40.1	14.2
9	17.8	9.8	17.7	14.5	15.8	-	10.4	18.5	20.0
10	9.2	9.9	12.2	16.2	15.2	13.3	20.8	18.8	34.2
11	11.9	13.3	10.1	14.0	20.6	14.0	18.2	-	13.1
12	8.3	8.9	15.3	13.0	13.5	11.8	13.0	-	13.6
13	9.0	13.5	10.4	13.1	14.5	13.8	13.3	15.6	14.0
n	*10*	*8*	*9*	*13*	*12*	*11*	*9*	*7*	*11*
Mean	**12.5**	**12.1**	**12.4**	**15.0**	**16.2**	**14.8**	**14.7**	**20.0**	**17.8**
SD	**3.7**	**4.1**	**2.8**	**3.5**	**3.3**	**3.3**	**3.2**	**10.0**	**5.9**

Table 4.11 Depth of crater of eroded lesions as measured by TMR (μm)

	Time of exposure, minutes								
Tooth number	**60**	**90**	**120**	**150**	**180**	**210**	**240**	**270**	**300**
1	5.0	6.3	5.0	7.4	-	19.3	22.0	-	18.4
2	-	-	5.6	11.0	13.6	18.4	-	-	26.0
3	-	5.1	-	12.1	7.9	9.8	-	-	-
4	7.8	-	-	9.8	11.1	15.2	-	19.3	20.8
5	1.6	-	-	7.8	9.0	10.9	-	11.2	18.4
6	-	6.1	-	9.1	14.5	17.1	18.4	-	22.0
7	1.0	-	7.1	8.6	8.8	14.0	14.9	16.7	17.5
8	1.2	-	7.3	8.6	10.8	11.3	16.9	23.4	23.1
9	-9.2	8.9	5.6	9.6	12.6	-	16.9	15.1	12.3
10	-2.3	5.2	5.6	6.8	8.6	12.6	6.0	9.6	-3.4
11	-1.9	2.7	8.6	6.9	3.8	10.7	10.2	-	18.1
12	-2.1	4.6	7.0	7.7	10.8	16.7	18.1	-	20.4
13	0.1	2.6	7.4	9.2	10.5	15.4	19.5	18.4	24.7
n	*10*	*8*	*9*	*13*	*12*	*11*	*9*	*7*	*11*
Mean	**0.1**	**5.2**	**6.6**	**8.8**	**10.2**	**14.3**	**15.9**	**16.2**	**18.2**
SD	**4.6**	**2.0**	**1.2**	**1.6**	**2.9**	**3.2**	**4.9**	**4.8**	**7.7**

Table 4.12 Loss of fluorescence of eroded lesions as measured by QLF (ΔQ, mm^2·% positive values)

	Time of exposure, minutes								
Tooth number	**60**	**90**	**120**	**150**	**180**	**210**	**240**	**270**	**300**
1	0.5	0.6	0.6	3.6	5.9	6.0	8.8	9.3	9.6
2	0.9	1.3	5.7	6.5	7.9	10.1	8.5	14.2	16.0
3	0.3	0.4	1.0	2.6	3.7	5.9	8.1	10.5	11.2
4	0.1	0.1	0.4	1.1	3.8	4.4	2.2	4.9	5.2
5	0	0.1	0.2	0.2	0.8	3.0	3.4	7.7	11.8
6	2.1	2.7	6.1	11.1	12.2	17.9	19.2	19.9	20.4
7	0	0.2	0.4	2.2	2.9	3.3	7.6	8.8	10.4
8	1.4	3.2	8.7	19.6	22.5	28.6	28.3	32.1	35.9
9	4.2	7.1	12.2	15.4	17.8	18.9	19.3	20.6	25.7
10	2.5	3.2	6.3	5.8	7.2	10.1	14.5	17.0	17.5
11	6.8	10.1	15.3	13.4	21.4	26.8	25.6	27.6	28.5
12	6.3	8.8	13.5	12.4	18.4	20.9	21.8	21.4	22.6
13	1.9	5.3	9.9	11.1	13.5	14.5	16.9	19.0	25.3
Mean	**2.1**	**3.3**	**6.2**	**8.1**	**10.6**	**13.1**	**14.2**	**16.4**	**18.5**
SD	**2.3**	**3.5**	**5.4**	**6.2**	**7.5**	**8.9**	**8.4**	**8.1**	**8.9**

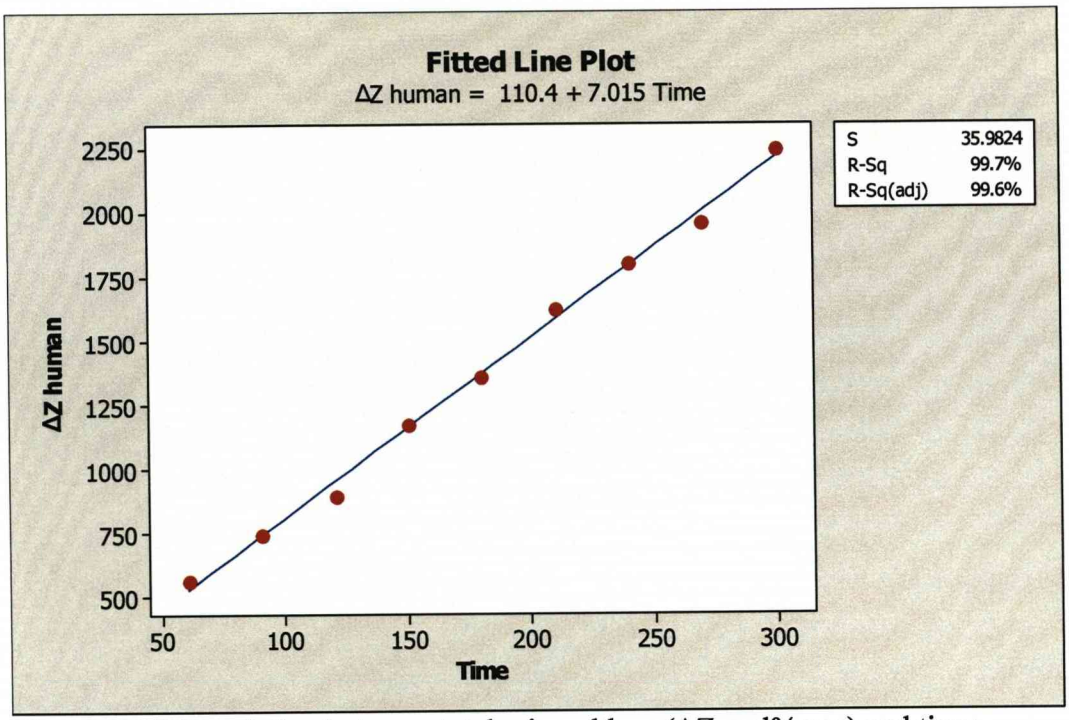


Figure 4.17 Correlation between total mineral loss (ΔZ, vol%·μm) and time (minutes) of erosive exposure in human enamel

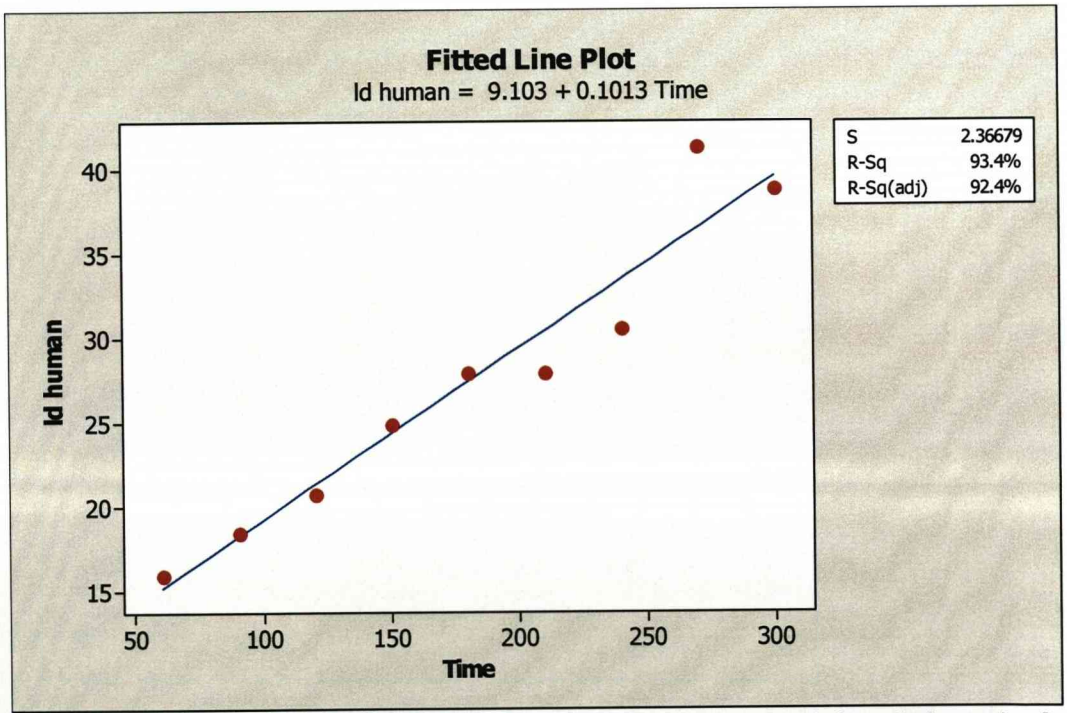


Figure 4.18 Correlation between total lesion depth (ld, μm) and time (minutes) of erosive exposure in human enamel

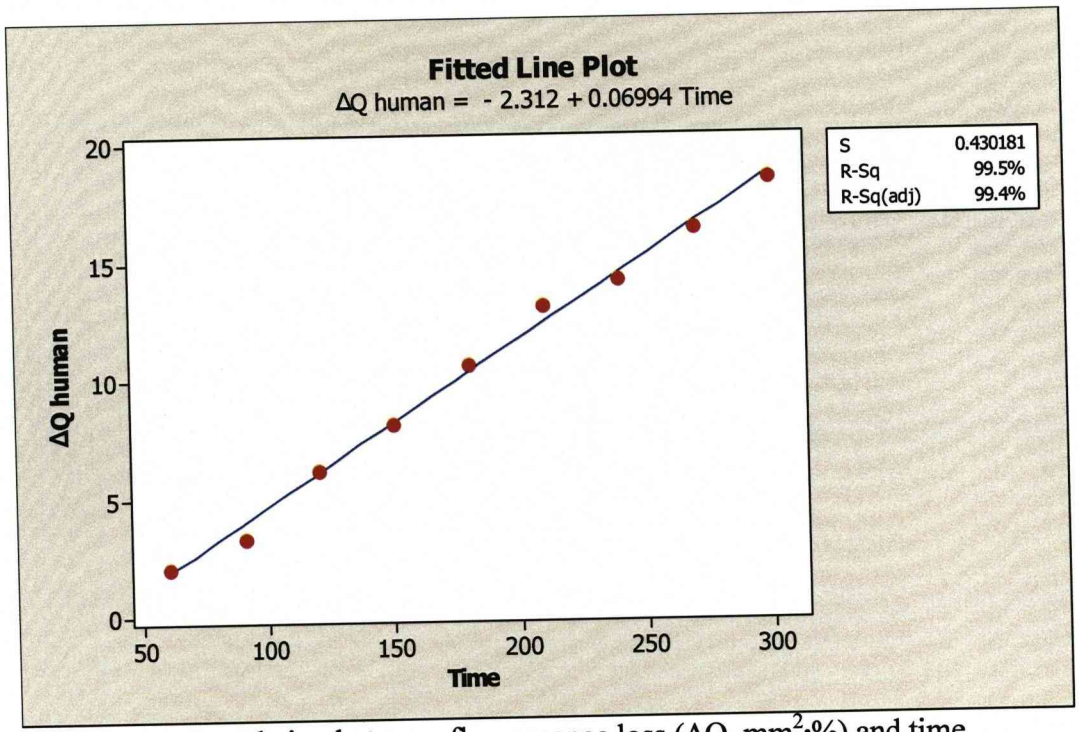


Figure 4.19 Correlation between fluorescence loss (ΔQ, mm^2·%) and time (minutes) of erosive exposure in human enamel

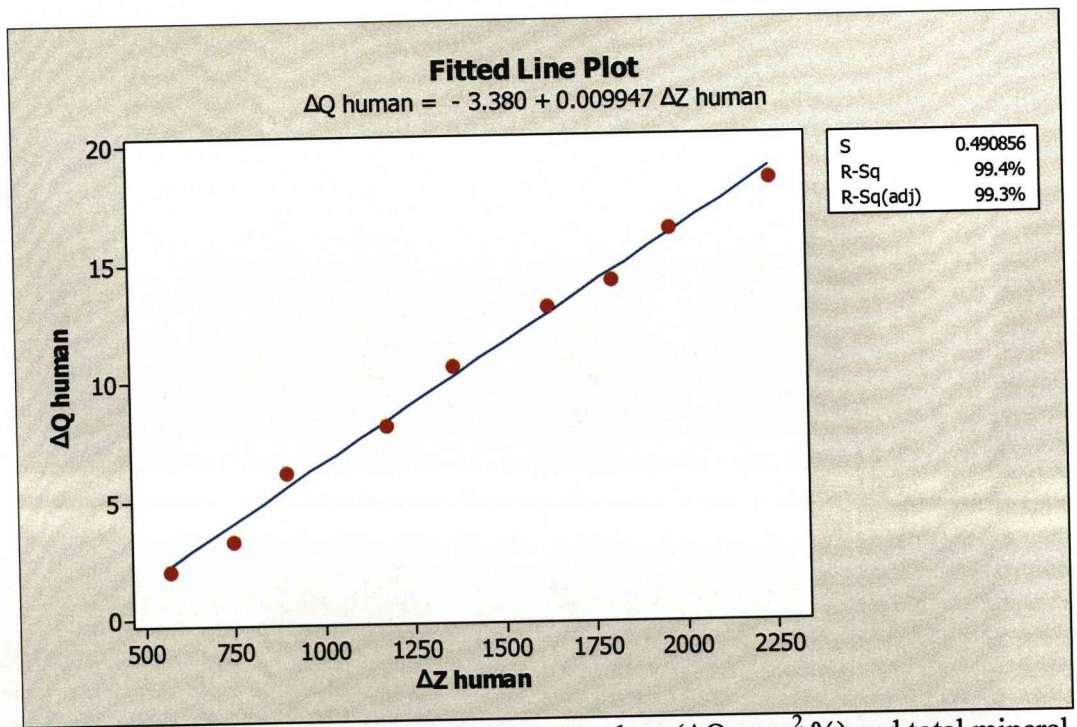


Figure 4.20 Correlation between fluorescence loss (ΔQ, mm^2·%) and total mineral loss (ΔZ, vol%·μm) of erosion in human enamel

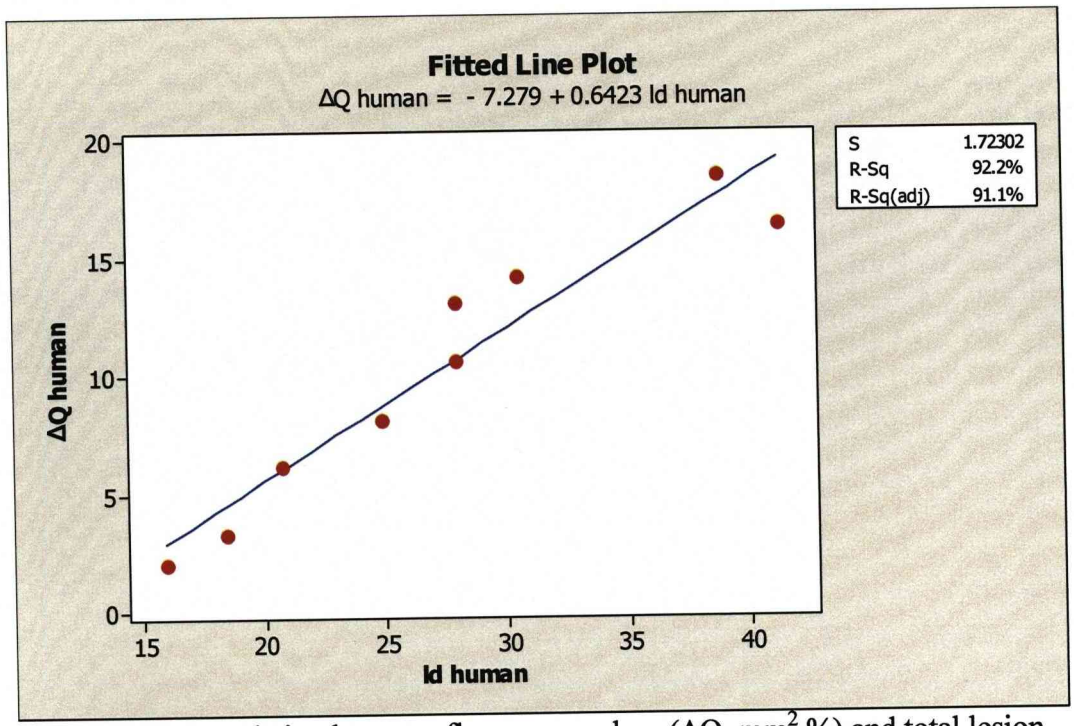


Figure 4.21 Correlation between fluorescence loss (ΔQ, mm^2·%) and total lesion depth (ld, μm) of erosion in human enamel.

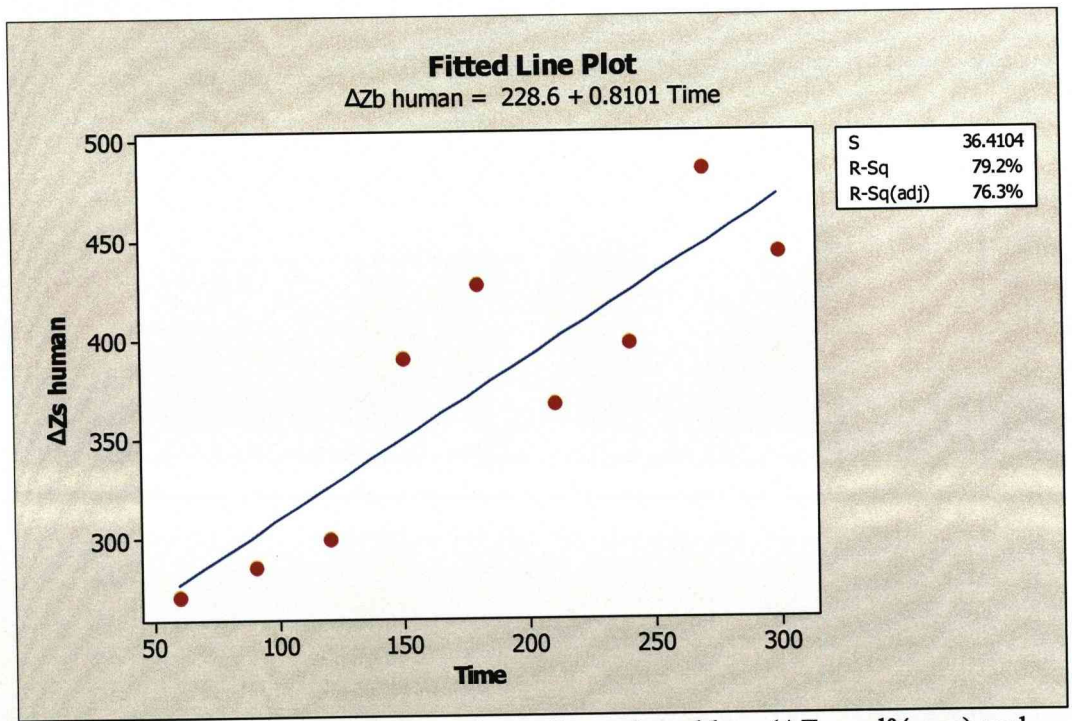


Figure 4.22 Correlation between only surface mineral loss (ΔZs, vol%·μm) and time (minutes) of erosive exposure in human enamel

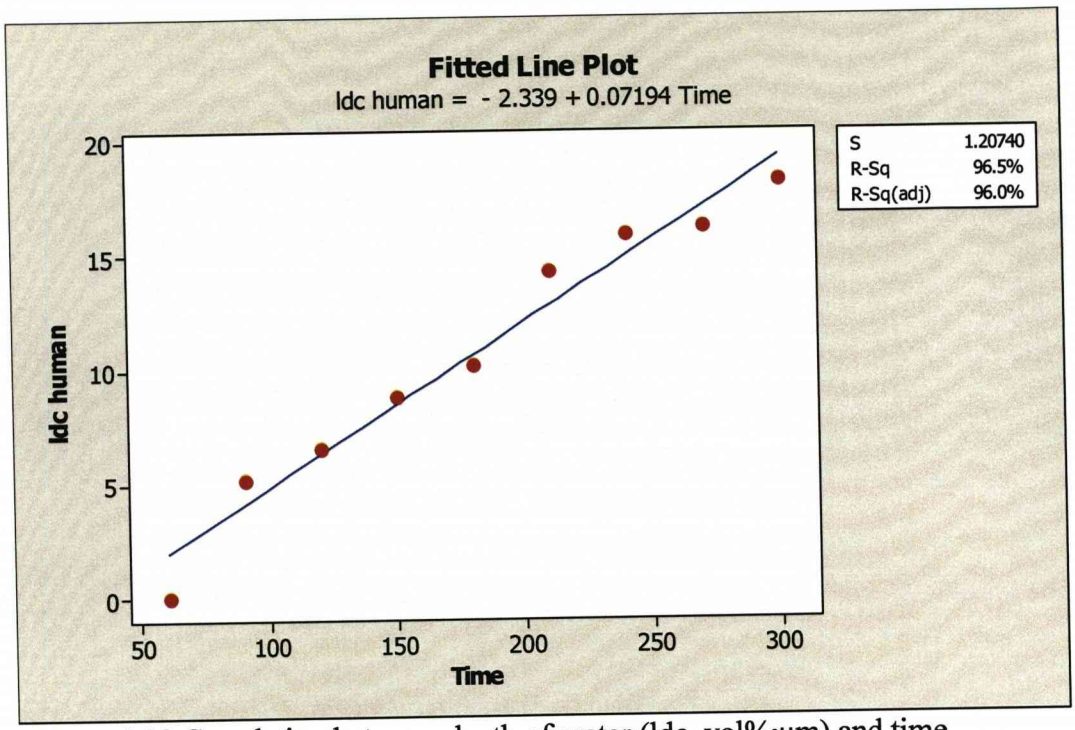


Figure 4.23 Correlation between depth of crater (ldc, vol%·µm) and time (minutes) of erosive exposure in human enamel

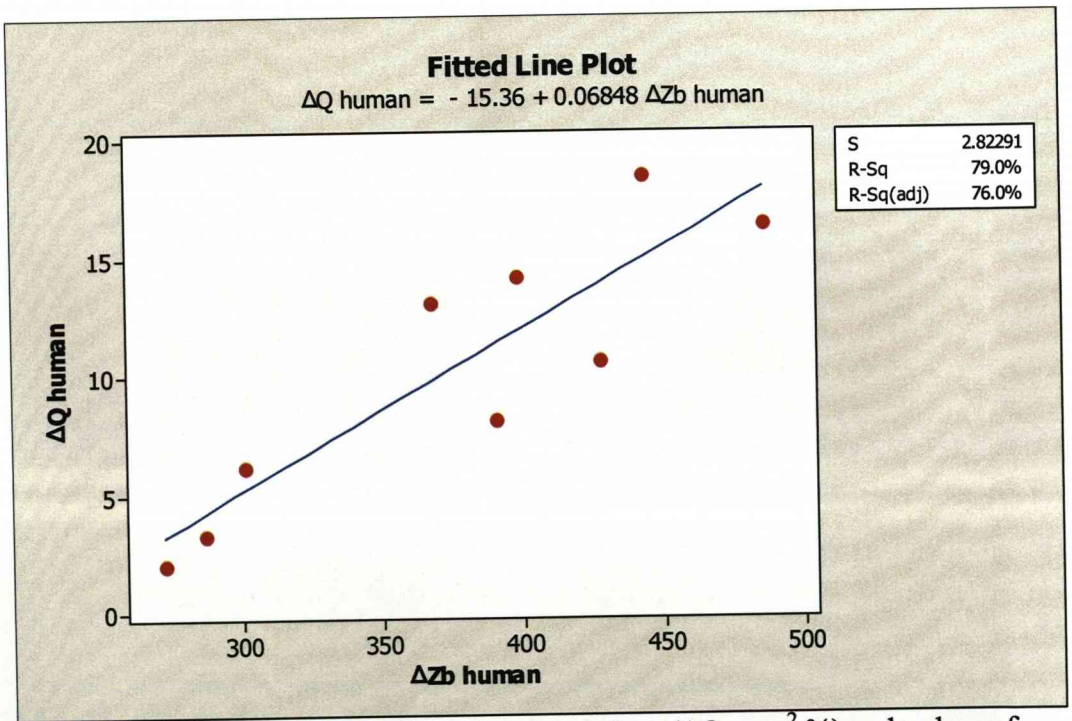


Figure 4.24 Correlation between fluorescence loss (ΔQ, mm^2·%) and only surface mineral loss (ΔZ, vol%·µm) of erosion in human enamel.

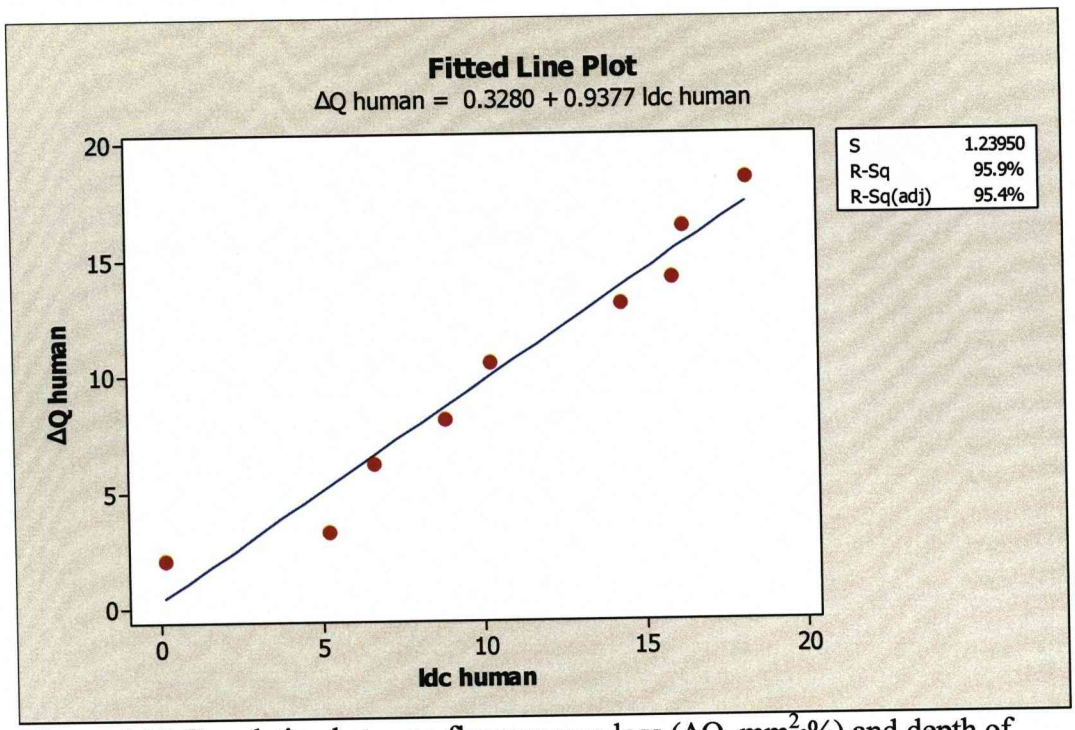


Figure 4.25 Correlation between fluorescence loss (ΔQ, $mm^2 \cdot \%$) and depth of crater of erosion (ldc, μm) in human enamel

4.5 Discussion

Over recent decades the incidence of dental decay has dropped in the general population (Murray, 1998) whilst the prevalence of toothwear, including dental erosion has worsened (Deery *et al*, 2000; Dugmore and Rock, 2004) and this may be related to the increased consumption of acidic soft drinks (British Soft Drink Association. Report of Seminar in Heidelberg, 1991). The lack of a number of reliable, precise and non-destructive tools particularly to measure the longitudinal progression of dental erosion, both *in vitro* and *in vivo* has led to the search for alternative methods of assessing dental erosion. Quantitative Light-induced Fluorescence is a useful technique to track the changes in mineral content in dental enamel lesions both artificial and *in vivo* and this principle may also be applied to the process of enamel demineralisation in the process of enamel erosion (Pretty *et al,* 2004).

This study reported in this thesis has demonstrated the ability of QLF to detect and longitudinally monitor the progression of artificially produced enamel erosion in both bovine and human teeth for up to 300 minutes. The fluorescence (ΔQ) of enamel for all samples gradually decreased with time of exposure to orange juice (adj.R^2 = 98.2% for bovine and 99.4% for human teeth) and strongly correlated with total mineral loss (adj.R^2 = 98.4% for bovine and 99.3% for human teeth), total lesion depth (adj.R^2 = 98.8% and 91.1% respectively) as measured by

transverse microradiography. These findings are in agreement with the findings of other workers in this field (Pretty *et al* 2004).

The lesions created on the enamel surface by acidic drinks were characterised by two distinctive components; firstly a crater of completely dissolved enamel and secondly a softened surface of hypomineralised enamel. Both of these features alone or in combination with each other could contribute to the ability of QLF to identify and monitor the artificially formed type of erosion studied during the above experiment.

In order to verify the suggested explanation for the ability of QLF to evaluate the depth of erosion (see above), additional analysis of obtained TMR data was performed and depth of crater (ldc) together with mineral loss (ΔZs) of the eroded surface was computed and statistically assessed against time of exposure to orange juice and against loss of fluorescence. The results demonstrated that cratering of the erosive lesion is possibly the main contributor to the loss of fluorescence of eroded enamel as increasing depth had a strong linear with time (adj.R^2 = 99.5% for bovine and 96.0% for human teeth). However, analysis of the demineralised surface showed that mineral loss of this softened enamel layer also changed longitudinally, but had reduced correlation with time of exposure. This tendency was noticed the same for both bovine and human samples used in this study (adj.R^2 = 76.3%). The similar trend was observed when loss of fluorescence (ΔQ) was plotted against crater depth (adj.R^2 = 98.4% for bovine and 95.4% for human teeth) and mineral loss of only surface of the lesion with less significant

correlation for latter (adj.R^2 = 78.9% and 76.0% respectively) for both bovine and human teeth. These results supported the findings by Pretty *et al* (2004) in their claim that the depth of the crater plays a major role in the ability of QLF to monitor the progression of dental erosion. In addition, the increased demineralisation of the surface of the eroded lesion also contributes to loss of fluorescence but to a lesser degree.

It could be speculated that the demineralised softened surface at the bottom of the crater is necessary to initiate scattering of light radiance, this could then be enhanced by the walls of the crater and this effect would be detected by means of QLF. However, in the absence of a softened base there would be insufficient scattering and therefore no measurable change in fluorescence. This situation, when cratering is present without softening of the base, resembles clinical observations reported in which erosion lesions have a smooth, glazed and rounded appearance said to follow frequent attacks by extrinsic and intrinsic acids (Bartlett and Smith, 2000). Dental erosion *in vivo* is usually combined with attrition and abrasion (Nunn, 2000), and it has been hypothesised that the primary superficial layer of enamel, which has been softened by acid attack, is subsequently removed by excessive rubbing forces derived from opposing teeth, tongue, cheeks, lips, food and possibly foreign objects placed into the mouth.

In the present study the constant cycling of human enamel in orange juice with a pH around 3.5 for 1 hour led to changes in fluorescence (delta Q) averaging only 2.1 ± 2.3 $mm^2 \cdot \%$ with a maximum value of 6.8 $mm^2 \cdot \%$. For two of the

samples that value was equal to zero. This observation could be explained by the possible natural variations in biological specimens. It is possible that some of the teeth selected for the study have had been exposed to fluoride to such an extent that their enamel structure had more resistance to acidic attack, such that lesions could not develop. The degree of *in vivo* surface demineralisation of enamel caused by brief (usually seconds) contact with acids is very small and possibly could not be detected using QLF. Clinically this acidic challenge which triggers initial demineralisation may be followed by longer periods of saliva remineralisation and/or abrasive wear of softened enamel causing complete surface loss and creation of a shallow crater without pronounced walls possessing a polished superficial enamel layer at the base of the eroded crater. Thus the design of this study may have restricted applications *in vivo,* further investigations, which attempt to replicate scenarios involving abrasion or attrition, may be helpful in elucidating the ability of QLF to analyse the effects of combined toothwear.

Transverse microradiography is a technique in which destruction of the experimental samples is required thus making longitudinal analysis of demineralised enamel impossible *in vitro* and *in vivo* (de Josselin de Jong *et al*, 1987). By comparison QLF provides one easy to use approach to study longitudinal changes *in vitro* enamel erosion experiments. It is a non-destructive technique and is notably less time consuming than TMR (ten Bosch, 1996; van der Veen and de Josselin de Jong, 2000). The findings of this study suggest that QLF may be an appropriate means to study dental erosion *in vitro*.

During the experiment involving human enamel samples some specimens were lost as a result of the mechanical processes used in preparing the samples for TMR, which involve cutting and grinding on a microscopic scale. With bovine teeth no such problems arose chiefly because of the larger dimensions of the teeth themselves and also because of the relatively flat surfaces created by the morphology of the teeth, which was used in calculations of regression coefficient. From this point of view the bovine teeth are preferred in studies with a similar design to the one reported in this thesis or perhaps when there is a lack of availability of human enamel samples. However, there is a need to consider the differences in morphological structure and in the rates of de- remineralisation between human and bovine enamel when devising experiments of this nature, as failing to do so could be misleading.

4.6 Conclusions

It was concluded that Quantitative Light-induced Fluorescence was able to detect, quantify and longitudinally monitor mineral loss due to active erosion *in vitro*. There was a powerful linear relationship between loss of fluorescence of bovine and human enamel with time of exposure to the erosive challenge.

When compared with the more established technique of TMR, QLF showed excellent correlation with depth of the eroded crater, while surface demineralisation at the base of the crater was also present.

QLF is an easy to use, non-destructive method and, may be a useful tool for assessment of mineral loss following erosive challenge *in vitro.* Further work would be necessary to evaluate the possibility of using QLF to detect and monitor erosion *in vivo*. The major advantage would be if the QLF technique would in same way be used in conjunction with a technique that would map the contours of the crater, for example, a non-contact profilometer for *in vivo* use.

The bovine teeth in this experiment demineralised more rapidly than human teeth in agreement with previous studies and may be explained by differences in porosity of their enamel and showed similar pattern of the development of erosive lesion. The bovine teeth are a comparable substitute to human teeth and due to their size and availability could be to the advantage of research community.

Chapter 5

THE EROSIVE PROPERTIES OF A NEWLY FORMULATED CALCIUM CONTAINING ACIDIC SOFT DRINK

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5.1 Introduction

As already stated elsewhere in this thesis toothwear is a well documented significant oral health problem of increasing prevalence in all age groups. Dietary acids play an important contributory role in the development of pathological toothwear and in dental erosion in particular (Birkhed, 1984; Lussi *et al*, 1991; Milosevic *et al*, 1997; Ganss *et al*, 1999). The global market of soft drinks is witnessing a continuing annual growth of 4-5%. Two thirds of this market belongs to carbonated and fruit juice containing drinks (Zenith International, 2006) and it is believed that they play the major role in increasing the prevalence of dental erosion in human population.

The composition and properties of soft beverages are of great interest since dental erosion has been associated with their consumption (Restarski *et al*, 1945a; Wynn and Haldi, 1948; Mistry and Grenby, 1993). Acids such as phosphoric, citric, malic, lactic and tartaric were found solely or in combination to be the most aggressive erosive agents in the composition of many drinks including fruit juices and colas (Rytömaa *et al*, 1988; Grando *et al*, 1996; Grenby *et al*, 1989; Lussi *et al*, 1995). These acids, naturally present or added to the beverages to enhance their composition, act as one of the most significant factors of flavour and taste as well as play a role in preserving the condition of drinks preventing microbiological spoilage. The most common beverages originated from juices and nectars and these are known to contain organic acids, such as citric, malic and tartaric acids

typically with concentrations between 0.3 and 6 % (w/v) and pH below 5 (West *et al*, 2000).

Phosphoric acid, due to its strength and low cost, is extensively used in the production of popular carbonated drinks, which account for more than 40% of all soft drinks consumed globally (Zenith International, 2003). Phosphoric is not an organic acid but is derived from minerals and is present in carbonated drinks, in particular 'cola' drinks, at a concentration of about 0.1% (w/v) (West *et al,* 2000).

The amount and frequency of soft drink consumption in the human diet and their potentially adverse effects on human teeth have led to increasing attention being paid to the possibility of modifying the composition of beverages in order to reduce their erosive effect. Several factors can affect the erosive potential of dietary acids on human teeth including type of acid, pH, titratable acidity, temperature, time of exposure, presence of other ions and substances such as sugar or sugar substitutes. The erosive potential of dietary drinks varies with the particular product under consideration and it has been shown that this harmful effect of soft drinks can be modified by altering the acidity of substances or using additives such as fluoride, calcium and phosphate ions as well as replacing sugars with sugar substitutes (Wagg *et al,* 1965; Reussner *et al,* 1975; Beiraghi *et al*, 1989; Larsen and Nyvad, 1999; Hughes *et al,* 1999a).

The increasing attention paid to the clinical effects of dental erosion suggests further research is warranted in order to find suitable methods for quantifying erosion *in vivo*, *in situ* and *in vitro,* which in turn can be used to further

elucidate potential preventive strategies in the management of this condition. Transverse microradiography has been a useful technique in erosion studies; however it is unfortunate that it is destructive and therefore neither suitable for *in vivo* studies nor for longitudinal monitoring of erosion. Recent data suggests that Quantitative Light-induced Fluorescence may be useful for assessing changes in mineral content of dental enamel following an erosive challenge (Pretty *et al*, 2004). In the previous chapter it was shown that QLF could detect and longitudinally monitor the progression of dental erosion both on bovine and human samples *in vitro* up to 5 hours.

5.2 Aims and objectives

5.2.1 Aim

The aim of this study was to evaluate *in vitro* the erosive potential of some commonly consumed soft drinks on sound and previously pre-eroded human enamel against a newly formulated soft drink containing added calcium and a negative control (pH neutral mineral water) using Transverse Microradiography and Quantitative Light-induced Fluorescence.

5.2.2 Objectives

The objectives of this study were to:

- investigate the erosive potential of several commonly consumed soft drinks;

- correlate QLF data of experimental eroded human enamel with the corresponding data obtained with transverse microradiography (TMR);

- investigate the effect of adding calcium to an acidic soft drink has on its ability to erode human enamel and compare this with the erosion seen with other commercially available soft drinks with erosive potential.

5.3 Materials and Methods

5.3.1 Tooth selection and initial preparation

110 human premolars without caries, cracks and malformation deformities were selected for the study. They were cleaned, pumiced with a toothbrush and buccal surfaces were polished with 1,200-grit sandpaper (Wet or Dry Sandpaper, 151 Products Limited, Manchester, UK). All samples were cut into half vertically through buccal and lingual surfaces. Both halves were painted with translucent acid resistant nail varnish (MaxFactor Nailfinity 101 Crystal Clear) except for 2 windows on buccal surface 2x3 mm.

5.3.2 Creation of initial erosive lesions

One half of each tooth was fixed on a cotton thread and suspended in 1% citric acid, pH 3.8, for 3 hours. The erosive lesions created were analysed with QLF version 1.99 (Inspektor Research Systems BV, The Netherlands). Only a few of the samples showed mineral loss by QLF. The teeth were subject for further erosive challenge in 1% citric acid for the next 3 hours. 15 teeth out of 111 with ΔQ values of 15 $mm^2 \cdot \%$ and above were selected for the study.

This series of experiments aimed to study the effects of erosion on both sound and pre-eroded enamel, so a methodology had to be devised to take account of these objectives. This required developing a means of producing reliable and consistent experimental erosion in enamel samples. This was attempted using 1% citric acid and evaluating the samples obtained by these methods using QLF and TMR. It became apparent that erosion by these experimental methods failed to produce sufficiently reliable lesions for analysis by QLF. Therefore given that the use of orange juice had in the past demonstrated its ability to produce consistent results for erosion lesions in enamel it was decided to use this to produce the pre-eroded lesions in this series of experiments.

For that reason the remaining teeth were exposed to orange juice (Tesco value, Tesco PLC, UK) for 2 hours. Adequate loss of fluorescence was detected in 42 samples using QLF.

30 additional human premolars were prepared for the study and painted with nail varnish except for a window of exposed enamel on the buccal surface. They were subject to the erosive challenge in orange juice for 3 hours, 25 more samples with acceptable erosive lesions were selected for the study.

5.3.3 Preparations for baseline analysis and group assignment

In order to obtain the baseline values for dental erosion, one edge of each lesion window on the teeth was marked with pencil and painted over with the same nail polish with the aim of conducting TMR analysis at the end of the experiment. After the varnish had dried the final QLF images were taken and analysed, excluding the one border of the analysing frame, which related to the marked side of the window.

All teeth were randomly assigned into 8 groups. Both halves of the teeth bearing windows with pre-eroded lesions and windows exposing the sound enamel were joined using low fusing dental compound (Green Stick Impression Compound, Kerr, Sybron, USA) and suspended on cotton threads in 50ml plastic beakers 5 teeth in each, 2 beakers per group and exposed to the testing solutions outlined below.

Group 1 – TO (orange juice, Tesco value, Tesco PLC, UK)

Group 2 – V (Volvic Natural Mineral water, Volvic, France)

Group 3 – CK (Coca-Cola, The Coca-Cola Company, USA)

Group 4 – D (Diet Coca-Cola, The Coca-Cola Company, USA)

Group 5 – J (sugar containing fruit drink SunnyD, Sunny Delight Beverages Company, USA)

Group 6 – S (no added sugar fruit drink SunnyD, Sunny Delight Beverages Company, USA)

Group 7 – C (fruit drink SunnyD California, Sunny Delight California, Sunny Delight Beverages Company, USA)

Group 8 – P (friut drink SunnyD containing added calcium in the form of calcium citrate malate, Sunny Delight Beverages Company, USA, Figure 5.1)

Figure 5.1 Drinks used in the experiment

5.3.4 Cycling procedure

All samples were stored at room temperature in artificial saliva and gently agitated. The saliva was changed every Monday and Thursday during the 4-week duration of the experiment.

The samples were subjected to testing solutions 3 times a day for 5 minutes Monday-Friday inclusive (Saturday and Sunday samples kept in artificial saliva) for 4 weeks. The testing drinks were taken from the fridge and were agitated during the erosive challenge. The samples were rinsed with deionised water before and after immersing in the test solutions. Twice a week on Monday afternoon and Thursday (Table 5.1) afternoon the teeth were rinsed, left to dry for 30 minutes on the bench and QLF images taken.

The final QLF images were taken at the end of the 4-week experiment. The QLF data were grouped and analysed.

Table 5.1 Collection dates of QLF data and corresponding time of erosive challenge.

	Week one		Week two		Week three		Week four		Week five
Day	Day 1 Monday, baseline	Day 2 Thursday	Day 3 Monday	Day 4 Thursday	Day 5 Monday	Day 6 Thursday	Day 7 Monday	Day 8 Thursday	Day 9 Monday, final
Erosion time, minutes	0	55	85	130	160	205	235	280	300

5.3.5 TMR analysis

The samples were cut into thin sections with a water-cooled diamond wire saw (Well, Walter Ebner, Switzerland) and were analysed using the TMR technique. Subsequently all the sections were mounted using nail varnish (MaxFactor®, Procter and Gamble, Weybridge, UK) to brass anvils and polished on both sides with a diamond disk to give plano-parallel specimens of 100 μm thickness. The sections were then mounted on a microradiographic plate-holder bearing an aluminium stepwedge (25 μm steps). The microradiographs were taken with a 15-min exposure on Kodak high-resolution plates (type 1A) using a Cu (Kα) X-ray source operating at 25 kV and 10 mA at a focus-specimen distance of 30 cm. The plates were developed using standard techniques. The microradiographs were subjected to image analysis under a Leica DMRB microscope (Leica, Germany). The image was captured at a magnification of 20x/0.40 via a CCD video camera (Sony, Japan) connected to a computer (Viglen PC, UK). The integrated mineral loss (vol%·μm), lesion depth (μm) and the depth of crater in control and experimental sections were assessed by a two-step image analysis technique by means of a software package (TMRW v. 1.22, Inspector research Systems BV, The Netherlands).

5.3.6 Chemical analysis of tested drinks

The pH of the drinks used in this experiment was tested at room temperature with a combination pH electrode (Orion, Boston, MA, USA) connected to an Alpha 500 pH meter (Aqua Scientific, Kent, UK). Titratable acid was determined as the volume (ml) of 1M NaOH required to raise the pH of 100 ml of the drink to pH 7.0. Concentration of Calcium was assessed using Atomic Absorption Spectrometer (SpectrAA 220, Varian Inc, CA, USA), and concentration of Phosphate – using Linear Readout Ultraviolet Spectrophotometer (Cecil CE272, Cecil Instruments LTD, Cambridge, UK.)

5.3.7 Statistical analysis

The data were analysed statistically using a one-way analysis of variance (ANOVA) and correlation analysis (Pearson correlation) with the assistance of SPSS software (SPSS Inc, Chicago, Illinois, USA).

5. 4 Results

5.4.1 Chemical Properties and Composition of soft drinks

The pH, titratable acid, concentration of calcium and phosphate of soft drinks used in this experiment are shown in Table 5.2

Table 5.2 Summary of chemical parameters for agents used in erosive experiment

Drink	**pH**	**Titratable acid, mmoles l^{-}**	**Calcium, ppm**	**Phosphate, ppm**
Orange Juice	3.62	83.0	78.96	103.34
Volvic water	7.13	Nil	3.28	0.39
Coca-Cola	3.06	20.3	3.04	252.32
Diet Coke	3.44	24.0	4.72	83.72
Drink J	3.56	57.5	5.44	26.66
Drink S	3.51	66.5	9.04	24.34
Drink C	3.71	83.0	2.52	15.33
Drink P	3.83	74.0	415.80	9.86

5.4.2 QLF data

The mineral loss was measured by using the QLF technique and was found to increase with increasing time in the erosive environment. A linear correlation was observed between ΔQ measured by QLF and time of exposure to orange juice and test drinks J, S, C for both pre-eroded and from sound tooth samples. Pearson correlation coefficient varied from 0.69 to 0.92 in pre-eroded (Table 5.3) and between 0.95 and 0.97 from sound teeth (Table 5.4). In groups challenged with test Drink P and Volvic water there was no such correlation observed (Pearson correlation coefficient varied from 0.24 to 0.63, Tables 5.3 and 5.4).

In groups exposed to both types of cola drink the correlation was marginal or not significant. Pearson correlation coefficient was calculated as -0.43 and 0.03 in pre-eroded (Table 5.3) and 0.25 and 0.69 from sound samples (Table 5.4). QLF was unable to detect changes in mineral loss of dental enamel in the samples that had been subjected to cola drinks, yet further results obtained from TMR showed significant loss of mineral content in these groups.

Table 5.3 The mineral loss (ΔQ values, $mm^2 \cdot \%$) for pre-eroded tooth samples with time (collection period in Days)

Group		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Pearson correl, r
TO	ΔQ	28.8	58.9	62.1	52.8	64.2	61.7	55.9	67.2	66.0	*0.69*
	SD	*16.0*	*17.2*	*18.8*	*21.1*	*17.2*	*18.9*	*22.6*	*28.7*	*20.6*	
V	ΔQ	32.2	52.4	53.0	41.9	49.2	45.8	50.6	47.3	43.7	*0.24*
	SD	*15.5*	*22.1*	*21.7*	*18.0*	*19.0*	*19.1*	*21.5*	*21.3*	*15.6*	
CK	ΔQ	26.7	58.2	41.9	29.1	31.7	33.4	22.1	25.8	32.9	*-0.43*
	SD	*15.5*	*17.9*	*19.0*	*17.8*	*15.2*	*25.6*	*23.0*	*19.5*	*21.8*	
D	ΔQ	25.5	47.2	47.5	36.6	50.3	43.3	34.1	33.0	41.4	*0.03*
	SD	*11.3*	*24.7*	*17.9*	*19.2*	*24.5*	*15.8*	*18.1*	*13.7*	*8.9*	
J	ΔQ	26.1	41.7	59.4	78.9	82.2	86.0	81.4	91.1	94.0	*0.92*
	SD	*10.3*	*17.0*	*30.8*	*35.7*	*39.3*	*39.5*	*37.2*	*41.7*	*43.9*	
S	ΔQ	27.6	37.1	52.2	62.1	62.3	59.9	59.6	71.7	68.6	*0.90*
	SD	*12.6*	*10.2*	*12.0*	*12.6*	*16.3*	*20.2*	*13.0*	*17.8*	*13.0*	
C	ΔQ	28.5	39.0	51.1	68.3	67.1	69.3	61.6	74.2	73.0	*0.88*
	SD	*9.6*	*17.8*	*20.9*	*18.5*	*19.3*	*16.3*	*19.1*	*21.2*	*21.6*	
P	ΔQ	28.8	32.8	41.0	51.6	46.1	49.1	46.8	44.0	42.7	*0.63*
	SD	*9.1*	*13.7*	*14.2*	*17.7*	*9.0*	*11.6*	*11.9*	*12.9*	*9.4*	

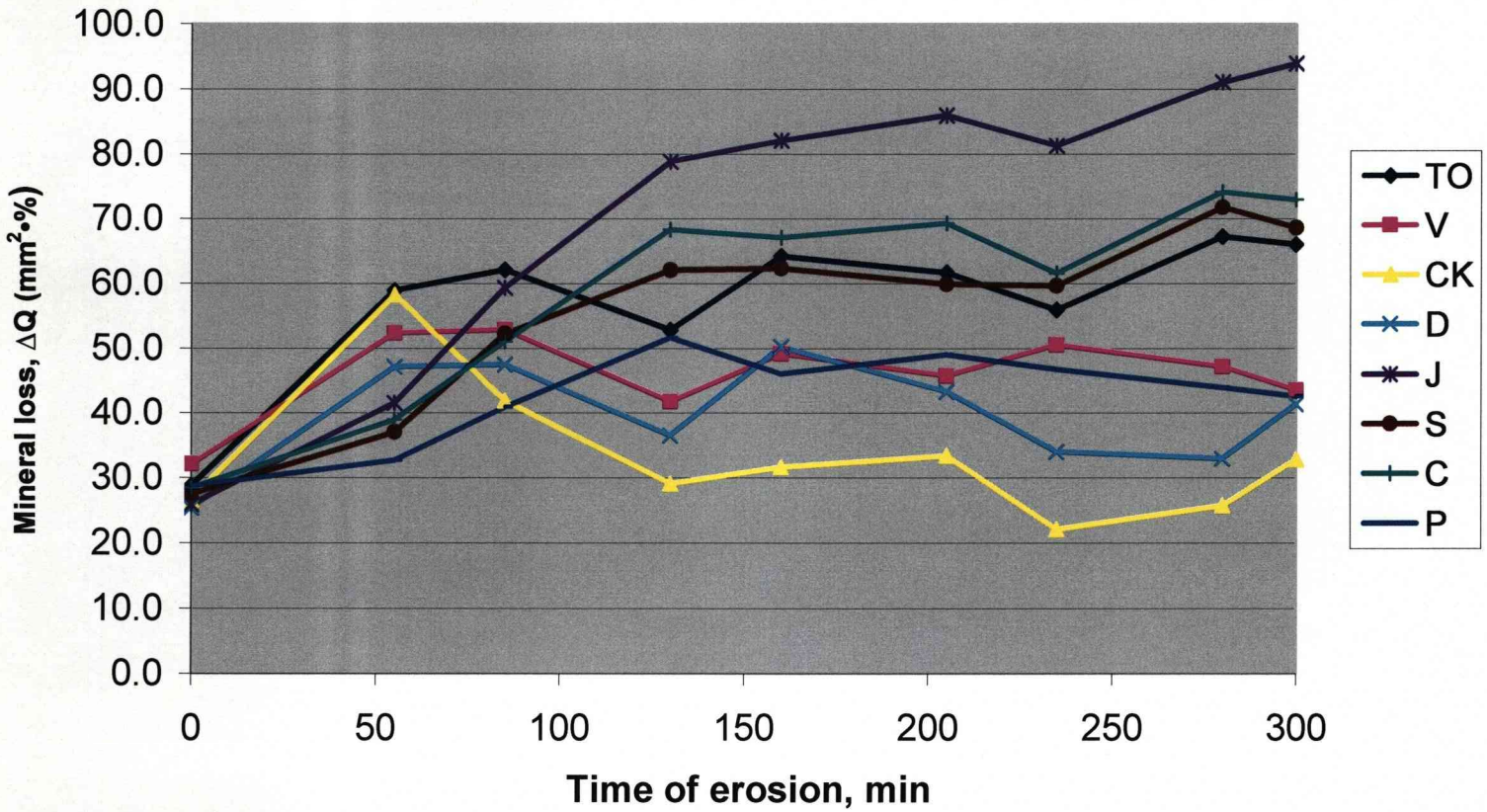


Figure 5.2 Changes in ΔQ for pre-eroded teeth within 8 study groups

Table 5.4 The mineral loss (ΔQ values, $mm^2 \cdot \%$) for from sound enamel with time (collection periods Days)

Group		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Pearson correl, r
TO	ΔQ	0.0	8.0	9.9	12.6	14.3	20.5	17.2	19.1	21.3	*0.95*
	SD		*8.3*	*10.9*	*13.6*	*7.9*	*18.4*	*14.9*	*15.2*	*19.0*	
V	ΔQ	0.0	12.6	12.7	6.3	6.9	8.9	8.2	10.6	8.2	*0.28*
	SD		*12.2*	*12.3*	*3.6*	*8.0*	*8.0*	*8.7*	*9.9*	*11.4*	
CK	ΔQ	0.0	51.6	21.0	16.6	24.6	26.8	23.7	25.1	32.7	*0.25*
	SD		*50.3*	*16.9*	*13.7*	*23.5*	*23.2*	*22.9*	*23.1*	*18.9*	
D	ΔQ	0.0	1.5	17.6	13.6	18.4	22.2	13.4	15.4	18.1	*0.69*
	SD		*1.4*	*14.1*	*11.9*	*14.8*	*18.7*	*10.6*	*11.6*	*15.6*	
J	ΔQ	0.0	8.1	24.1	59.5	65.7	69.8	73.1	89.9	95.9	*0.97*
	SD		*6.3*	*16.2*	*23.1*	*27.0*	*33.6*	*39.7*	*48.0*	*48.7*	
S	ΔQ	0.0	15.1	36.6	64.4	73.4	92.4	90.5	98.2	99.1	*0.97*
	SD		*13.0*	*28.1*	*37.9*	*39.6*	*50.9*	*50.1*	*54.9*	*49.1*	
C	ΔQ	0.0	6.5	23.8	52.7	54.7	63.1	61.7	77.3	74.4	*0.96*
	SD		*6.3*	*14.0*	*20.5*	*20.6*	*25.0*	*23.7*	*24.6*	*22.9*	
P	ΔQ	0.0	1.9	5.4	8.3	5.3	6.1	3.8	6.9	1.9	*0.34*
	SD		*1.8*	*9.2*	*13.0*	*7.5*	*9.7*	*4.4*	*12.0*	*1.6*	

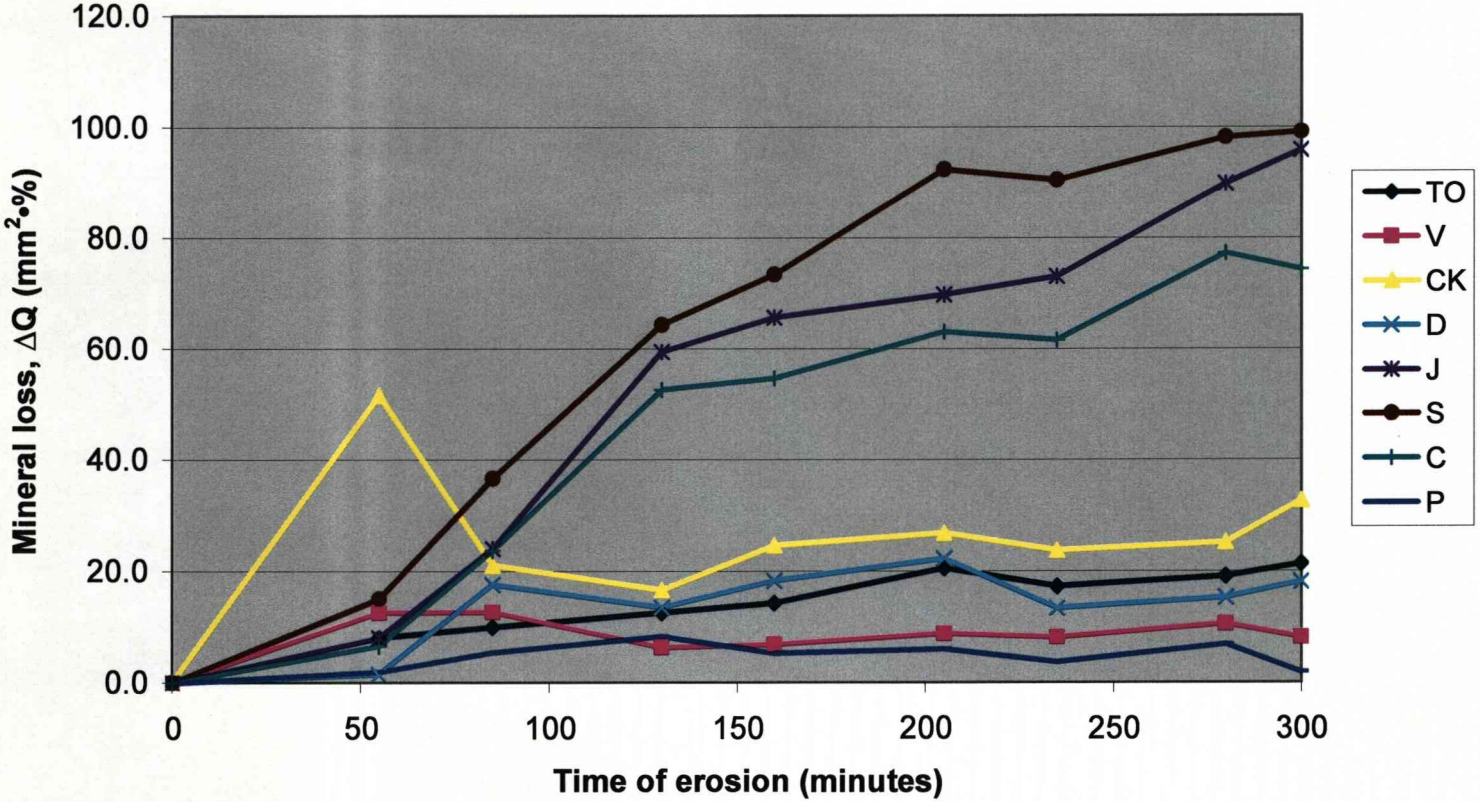


Figure 5.3 Changes in ΔQ for from sound enamel for 8 study groups

5.4.3 TMR data

The analysis of TMR data showed the increase of mineral loss and lesion depth in pre-eroded samples exposed to all tested drinks except Volvic water and Drink P. On the surface of the sound samples formation of new erosive lesions was detected with a similar exception as above.

5.4.3.1 Total mineral loss and total lesion depth

There was observed a significant difference in total mineral loss, ΔZ (Tables 5.5 & 5.6) and lesion depth (Tables 5.7 & 5.8) between all groups (one-way ANOVA, $p<0.001$) in both pre-eroded and from sound teeth samples with the highest mineral loss noticed in the Coca-Cola group.

Table 5.5 Total mineral loss, ΔZ (vol%·μm) in pre-eroded teeth

	Groups							
Tooth number	**Group 1 TO**	**Group 2 V**	**Group 3 CK**	**Group 4 D**	**Group 5 J**	**Group 6 S**	**Group 7 C**	**Group 8 P**
1	634.8	-192.8	3607.2	1704.6	2424.9	1534.7	1790.8	-181.5
2	1946.0	-779.6	5040.9	1085.9	3547.4	1548.2	1828.7	85.8
3	884.1	-441.1	2885.5	481.0	1842.4	2746.5	1408.1	175.8
4	1682.5	-564.7	2730.2	2519.4	2537.4	783.1	1781.3	75.8
5	2041.2	-140.8	3200.6	900.8	3505.1	2492.3	19.1	159.6
6	555.6	-477.2	1756.4	1806.1	1251.6	2184.8	1209.9	4694.2
7	1271.7	-747.4	2312.9	1236.7	1522.6	1971.0	1083.8	58.2
8	524.0	-59.7	3192.1	563.8	2582.8	2964.6	2335.6	12.8
9	590.8	37.4	1929.6	1545.8	2303.4	2653.0	797.5	-798.1
10			2029.2	2301.2	1816.1			130.6
Mean	**1125.6**	**-374.0**	**2868.5**	**1414.5**	**2333.4**	**2097.6**	**1361.6**	**441.3**
SD	**624.0**	**298.1**	**979.3**	**688.0**	**767.1**	**707.8**	**684.6**	**1522.0**

Significant difference between all groups, $p < 0.001$ (one-way ANOVA) at the 5% level.

"+" figures in this table mean that further demineralisation occurred in corresponding samples, while "-" figures point to mineral gain thus possible remineralisation

Table 5.6 Total mineral loss, ΔZ (vol%·μm) from sound teeth

Tooth number	Groups							
	Group 1 TO	Group 2 V	Group 3 CK	Group 4 D	Group 5 J	Group 6 S	Group 7 C	Group 8 P
1	808.3	231.8	4446.6	1401.2	2609.5	2353.5	1740.1	192.1
2	1255.7	233.8	3839.2	1511.3	2358.2	2206.7	2120.0	104.3
3	494.0	226.1	4334.5	1902.6	2685.7	2977.9	2810.6	59.5
4	819.3	257.6	4270.9	1834.7	2415.9	2148.2	2121.0	71.6
5	1193.6	330.9	3335.7	1655.5	2679.1	1920.5	1472.5	468.4
6	931.2	304.3	3498.8	1690.2	2243.9	2453.0	1703.8	381.8
7	1153.8	225.0	3966.7	1487.3	2521.1	1866.8	1972.1	253.7
8	1009.6	135.0	4605.0	1868.8	1811.8	2260.2	1964.2	251.6
9	857.3	181.5	3529.1	1373.6	2192.3	1857.6	1537.4	257.0
10	702.4	117.0	4091.9	2178.8	1545.4	2202.9	1561.1	793.2
Mean	**922.5**	**224.3**	**3991.8**	**1690.4**	**2306.3**	**2224.7**	**1900.3**	**283.3**
SD	**237.0**	**67.0**	**434.6**	**257.1**	**376.6**	**333.2**	**398.5**	**221.6**

Significant difference between all groups, $p < 0.001$ (one-way ANOVA) at the 5% level.

"+" figures in this table mean that further demineralisation occurred in corresponding samples, while "-" figures point to mineral gain thus possible remineralisation

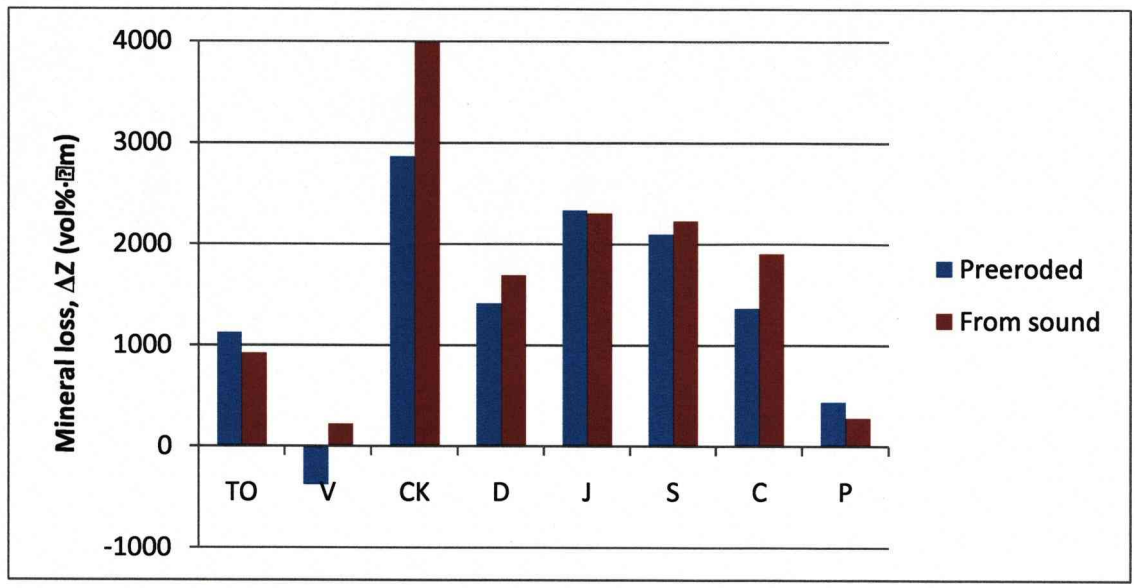


Figure 5.4 Total mineral loss, ΔZ (vol%·μm)

Table 5.7 Total lesion depth, μm in pre-eroded teeth

	Groups							
Tooth number	**Group 1 TO**	**Group 2 V**	**Group 3 CK**	**Group 4 D**	**Group 5 J**	**Group 6 S**	**Group 7 C**	**Group 8 P**
1	12.1	0.9	20.0	15.5	34.6	29.9	18.4	-10.1
2	32.8	-12.1	46.0	11.1	38.2	28.9	20.2	3.4
3	13.5	-0.1	21.7	6.4	38.4	37.3	11.3	-10.5
4	25.1	-12.2	26.7	33.5	51.6	21.5	31.6	7.0
5	24.7	21.4	27.1	10.3	52.2	35.2	10.0	0.9
6	9.1	-8.3	11.3	19.7	19.4	23.6	11.5	41.3
7	11.7	-11.1	22.9	6.2	23.4	28.4	15.1	4.6
8	2.6	-2.2	30.6	8.0	32.9	42.9	36.7	5.7
9	-0.9	0.5	13.2	19.8	26.2	41.6	13.4	-11.7
10			12.9	24.2	29.8			2.2
Mean	**14.5**	**-2.6**	**23.2**	**15.5**	**34.7**	**32.1**	**18.7**	**3.3**
SD	**11.0**	**10.5**	**10.4**	**8.9**	**11.0**	**7.6**	**9.5**	**15.1**

Significant difference between all groups, $p < 0.001$ (one-way ANOVA) at the 5% level.

"+" figures in this table mean that further demineralisation occurred in corresponding samples, while "-" figures point to mineral gain thus possible remineralisation

Table 5.8 Total lesion depth, μm from sound teeth

Tooth number	Groups							
	Group 1 TO	Group 2 V	Group 3 CK	Group 4 D	Group 5 J	Group 6 S	Group 7 C	Group 8 P
1	17.2	11.4	59.7	22.4	53.6	53.5	44.0	8.6
2	28.4	12.9	52.7	22.0	53.3	53.1	45.4	4.2
3	16.4	8.3	59.9	32.6	61.5	53.4	66.6	7.6
4	19.6	12.1	60.0	28.4	51.1	46.4	44.1	5.3
5	23.6	13.7	44.0	25.9	54.4	46.6	39.2	12.7
6	20.2	14.4	50.8	31.4	49.0	48.4	41.6	15.6
7	22.2	7.7	57.7	24.9	58.9	46.2	46.1	8.1
8	19.4	7.1	58.4	31.6	51.7	43.3	48.1	9.9
9	15.0	9.4	51.1	22.9	52.0	45.0	43.1	8.8
10	15.1	5.6	54.5	35.5	45.1	51.3	40.1	20.6
Mean	**19.7**	**10.3**	**54.9**	**27.8**	**53.1**	**48.7**	**45.8**	**10.1**
SD	**4.2**	**3.0**	**5.3**	**4.8**	**4.6**	**3.8**	**7.8**	**4.9**

Significant difference between all groups, $p < 0.001$ (one-way ANOVA) at the 5% level.

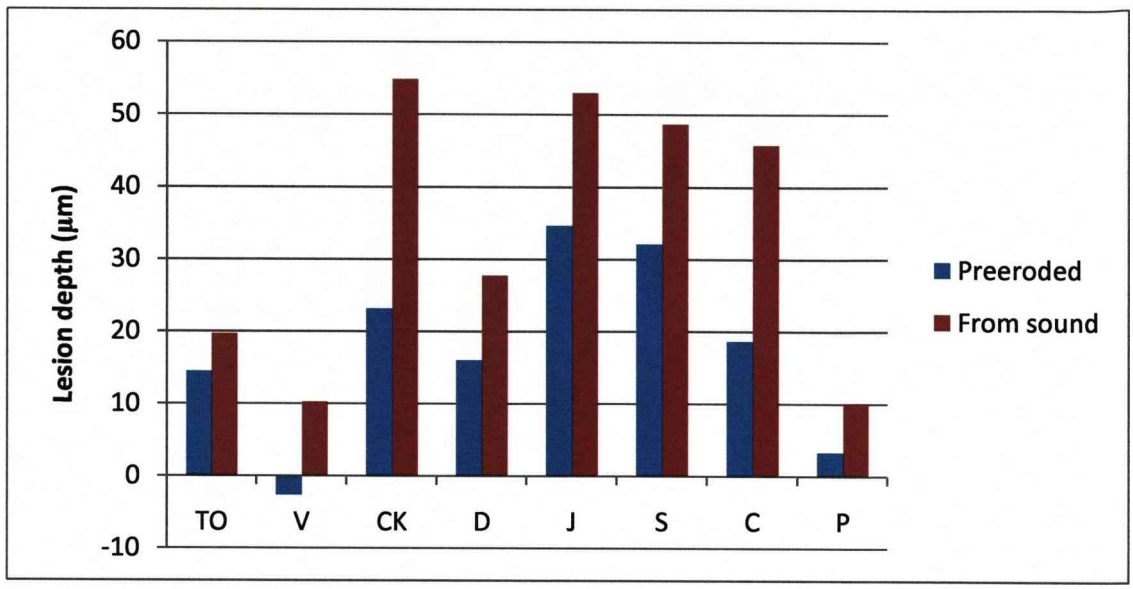
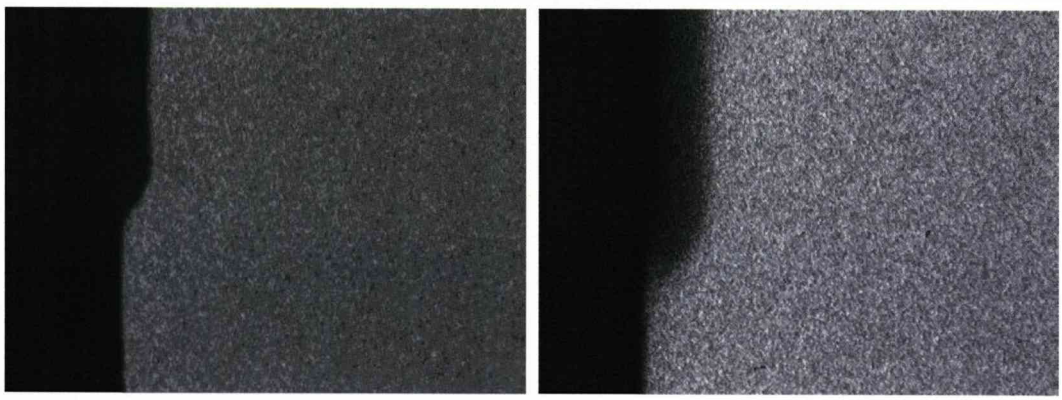


Figure 5.5 Total lesion depth, µm

5.4.3.2 Mineral loss and lesion depth at the surface of lesions

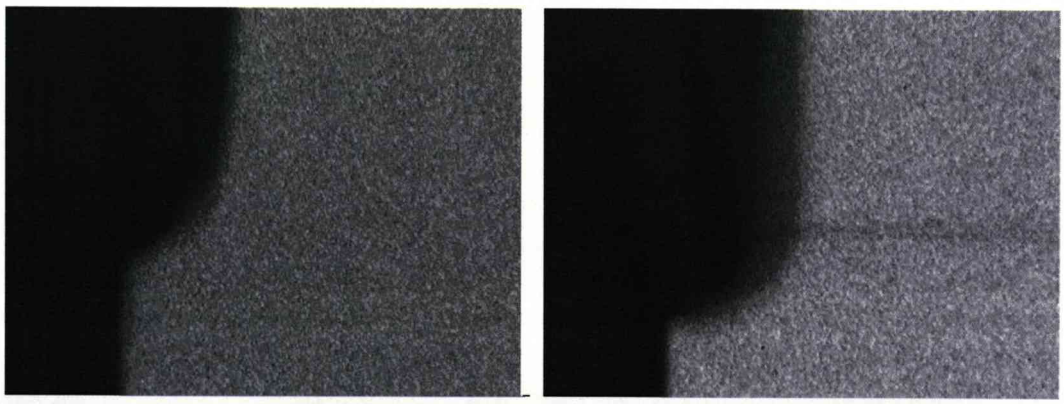
While total mineral loss and total lesion depth represent the total amount of minerals dissolved from dental enamel during erosive challenge and overall severity of the lesion they do not always provide a complete picture regarding the potential for possible remineralisation of eroded lesions. Figures 5.6 and 5.7 illustrate the variations of erosion: A – complete dissolution of enamel on the surface of the tooth, creating a deep or very deep crater, which is virtually impossible to restore by natural or forced remineralisation; B – shallow or deep

crater with profound surface softening, when the matrix of enamel hydroxyapatites remained and which could be repaired by means of remineralisation.



A B

Figure 5.6 Examples of transverse microradiographs illustrating two different types of the formation of mild erosive lesions.



A B

Figure 5.7 Examples of transverse microradiographs illustrating two different types of the formation of extensive dental erosion.

The following graphs (Figures 5.8 & 5.9) show the mineral loss and lesion depth of the actual surface of lesion but do not take into account the amount of mineral lost by the crater of lesion itself. Only at the surface of the lesions was there observed a significant difference in mineral loss (Tables 5.9 & 5.10) and lesion depth (Tables 5.11 & 5.12) between all groups (one-way ANOVA, $p<0.001$) in both pre-eroded and from sound teeth samples with the highest demineralisation noticed in the groups 5, 6 and 7 (SunnyD drinks J, S and C respectively).

It is clearly seen that in test groups J, S and C both parameters significantly exceed those corresponding to other erosive groups: Orange juice and both types of Coca-Cola for both pre-eroded and from sound samples ($p<0.001$). These data statistically support the assumption that erosion caused by SunnyD drinks J, S and C could possibly be remineralised in contrast to other tested erosive drinks.

Table 5.9 Mineral loss, ΔZ (vol%·μm) in pre-eroded teeth at the surface of lesions

	Groups							
Tooth number	**Group 1 TO**	**Group 2 V**	**Group 3 CK**	**Group 4 D**	**Group 5 J**	**Group 6 S**	**Group 7 C**	**Group 8 P**
1	77.4	91.1	-166.2	142.3	1021.8	1065.4	522.7	-155.4
2	331.6	-58.3	-352	-20.3	1165.0	768.9	-44.1	80.2
3	167.5	71	-278	66.1	1271.7	751.2	105.3	-263.9
4	390.7	-165.7	-42.1	407.4	1182.3	618.3	604.0	316.4
5	174.2	267.1	-140.6	29.3	770.2	864.7	503.9	140.2
6	118.8	23.1	-287.5	-88	659.8	261.9	582.1	62.8
7	70.3	117.5	-158.6	-71.2	634.4	497.6	411.0	39.3
8	-21.2	-112	-310.6	118.8	393.8	971.6	439.6	1.9
9	-61.5	-161.8	-207.4	37.8	862.8	519.2	565.5	-146.4
10			-264	-21.1	892.8			28.5
Mean	**138.6**	**8.0**	**-220.7**	**60.1**	**885.5**	**702.1**	**410.0**	**10.4**
SD	**148.9**	**144.9**	**94.4**	**143.2**	**279.6**	**253.5**	**227.2**	**165.6**

Significant difference between all groups, $p < 0.001$ (one-way ANOVA) at the 5% level.

"+" figures in this table mean that the area of softened surface became less mineralised in corresponding samples, while "-" figures point to increased mineralisation of lesion surface.

Table 5.10 Mineral loss, ΔZ (vol%·μm) from sound teeth at the surface of lesions

	Groups							
Tooth number	**Group 1 TO**	**Group 2 V**	**Group 3 CK**	**Group 4 D**	**Group 5 J**	**Group 6 S**	**Group 7 C**	**Group 8 P**
1	272.6	231.8	390.2	242.1	1567.2	1397.7	1123.6	192.1
2	527.7	233.8	352	175.7	1388.4	1150.5	1182.0	104.3
3	303.9	226.1	387.8	343	1497.5	1336.7	1200.5	59.5
4	336.9	257.6	411.9	260.4	1429.3	1410.6	1249.5	71.6
5	348.2	330.9	334.8	244.8	1273.8	1268.8	996.7	240.9
6	335.4	304.3	401	342.5	1480.7	1166.0	1076.4	251.3
7	386.9	225	371.2	281.2	1385.6	1080.5	1126.3	219.1
8	291	135	259.7	335.5	1328.9	1079.7	1207.8	251.6
9	184.2	181.5	431.1	202.4	1463.7	1314.0	1128.3	257.0
10	246	117	432.5	290.4	1061.2	1384.8	1089.4	444.8
Mean	**323.3**	**224.3**	**377.2**	**271.8**	**1387.6**	**1258.9**	**1138.0**	**209.2**
SD	**91.9**	**67.0**	**51.9**	**58.1**	**142.8**	**129.8**	**74.3**	**113.1**

Significant difference between all groups, $p < 0.001$ (one-way ANOVA) at the 5% level.

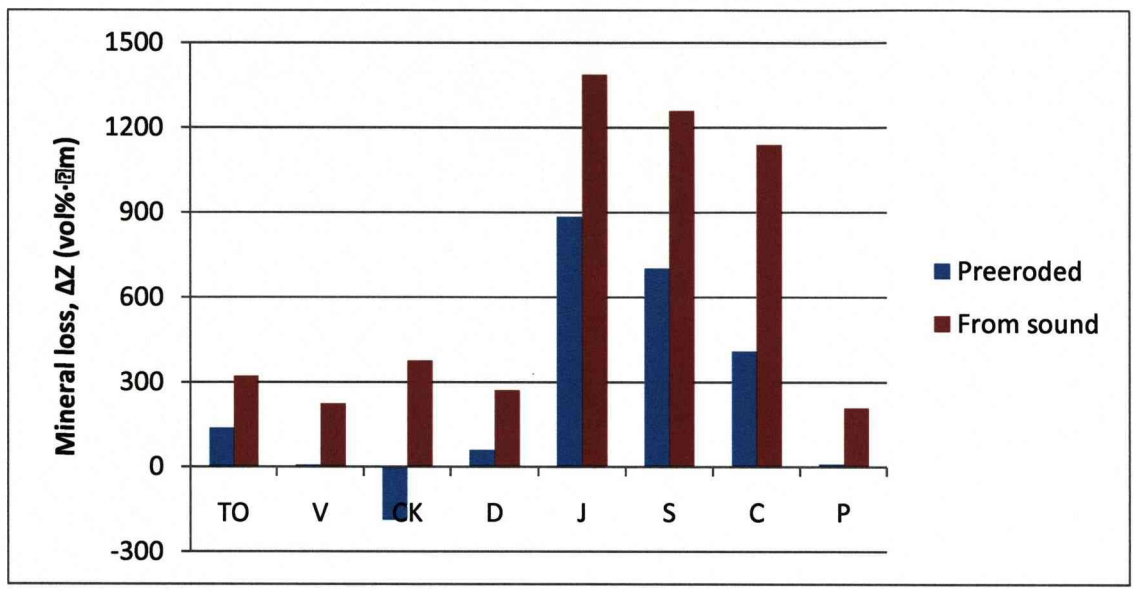


Figure 5.8 Mineral loss at the surface of erosion, vol%·μm

Table 5.11 Lesion depth, μm in pre-eroded teeth at the surface of lesions

	Groups							
Tooth number	**Group 1 TO**	**Group 2 V**	**Group 3 CK**	**Group 4 D**	**Group 5 J**	**Group 6 S**	**Group 7 C**	**Group 8 P**
1	5	3.9	-16.8	-1.2	18.9	24.9	6.0	-10.3
2	13.5	-2.4	-14.4	-1	10.6	22.6	-3.6	2.9
3	5.2	5.5	-14.9	3.2	31.0	14.7	-3.9	-15.5
4	9.6	-7.1	-3.3	9.9	36.1	20.2	16.7	8.9
5	4.3	26	-8.7	0.5	15.2	17.6	13.9	-0.7
6	4.7	-2.8	-10.8	-3	11.6	2.4	7.7	-4.2
7	-1.5	0.4	-5.7	-8.4	13.1	9.8	8.6	3.5
8	-3	-2.8	-11.3	2.6	5.8	18.6	11.8	5.3
9	-6.2	-5	-9.1	0.7	9.0	14.2	9.8	-4.0
10			-13.6	-1.3	18.7			1.7
Mean	**3.5**	**1.7**	**-10.9**	**0.2**	**17.0**	**16.1**	**7.4**	**-1.2**
SD	**6.2**	**9.9**	**4.3**	**4.7**	**9.7**	**6.9**	**7.1**	**7.4**

Significant difference between all groups, $p < 0.001$ (one-way ANOVA) at the 5% level.

"+" figures in this table mean that the area of softened surface became wider in corresponding samples, while "-" figures point to decrease of the lesion depth of the softened surface.

Table 5.12 Lesion depth, μm from sound teeth at the surface of lesions

	Groups							
Tooth number	**Group 1 TO**	**Group 2 V**	**Group 3 CK**	**Group 4 D**	**Group 5 J**	**Group 6 S**	**Group 7 C**	**Group 8 P**
1	10.5	10.5	13.9	8.7	40.8	41.8	36.4	8.6
2	19.7	19.7	13.3	6.9	41.4	39.3	34.0	4.2
3	13.7	13.7	15.7	14.6	46.8	33.9	46.5	7.6
4	13.7	13.7	16.3	10.2	39.2	37.6	33.4	5.3
5	13.6	13.6	11.2	9.5	37.3	38.4	33.4	9.9
6	13.2	13.2	15.4	15.8	39.0	33.1	34.0	13.8
7	13.5	13.5	15.9	10.9	44.8	36.3	35.1	7.7
8	11.6	11.6	9.4	14	45.4	28.4	38.8	9.9
9	7.2	7.2	16.4	9.7	43.1	38.1	37.8	8.8
10	9.6	9.6	13.8	13.9	39.0	41.1	34.3	16.2
Mean	**12.6**	**12.6**	**14.1**	**11.4**	**41.7**	**36.8**	**36.4**	**9.2**
SD	**3.3**	**3.3**	**2.3**	**3.0**	**3.2**	**4.1**	**4.0**	**3.6**

Significant difference between all groups, $p < 0.001$ (one-way ANOVA) at the 5% level.

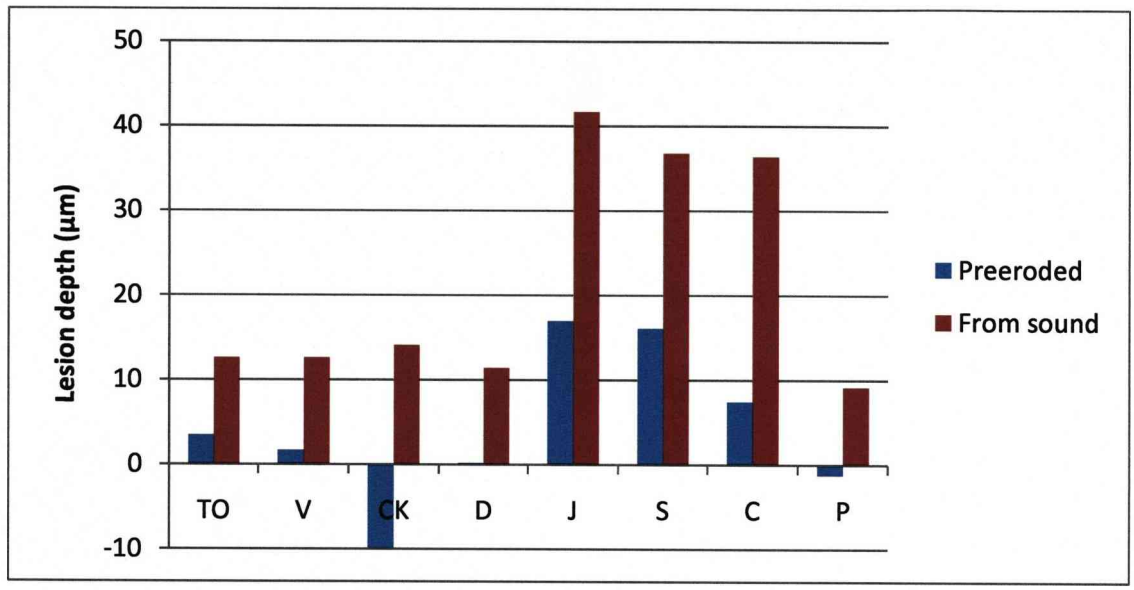


Figure 5.9 Lesion depth at the surface of erosion, µm

5.4.3.3 Depth of crater

The analysis of the depth of crater from sound samples (Tables 5.13 & 5.14) showed significant differences (one-way ANOVA, $p<0.001$) between all groups (Figure 5.10). Tukey multiple comparison test showed significant differences between the Coca-Cola and all SunnyD groups ($p < 0.001$); between Diet Coca-Cola and groups J ($p < 0.05$), C ($p < 0.01$), P ($p < 0.001$).

In pre-eroded teeth the difference was also significant between all 8 groups ($p<0.001$) but the Tukey multiple comparison test demonstrated significant differences only between only Coca-Cola and each SunnyD groups ($p<0.01$).

Table 5.13 Depth of crater, μm in pre-eroded teeth

	Groups							
Tooth number	**Group 1 TO**	**Group 2 V**	**Group 3 CK**	**Group 4 D**	**Group 5 J**	**Group 6 S**	**Group 7 C**	**Group 8 P**
1	7.1	-3.0	36.8	16.6	15.8	5.0	12.4	0.2
2	19.2	-9.7	60.4	12.1	27.6	6.3	23.8	0.4
3	8.3	-5.6	36.6	3.3	7.4	22.6	15.1	5.1
4	15.5	-5.2	29.9	23.6	15.5	1.4	14.9	-1.9
5	20.4	-4.6	35.8	9.8	37.0	17.6	-3.9	1.7
6	4.5	-5.5	22.1	22.6	7.8	21.2	3.8	45.5
7	13.2	-11.5	28.5	14.5	10.3	18.6	6.6	-6.1
8	5.5	0.6	41.9	5.4	27.1	24.3	24.9	0.4
9	5.4	5.5	22.3	19.0	17.2	27.4	3.6	-7.7
10			26.5	25.4	11.1			0.6
Mean	**11.0**	**-4.3**	**34.1**	**15.2**	**17.7**	**16.0**	**11.2**	**3.8**
SD	**6.2**	**5.1**	**11.3**	**7.6**	**9.8**	**9.4**	**9.6**	**15.1**

Significant difference between all groups, $p < 0.001$ (one-way ANOVA) at the 5% level.

"+" figures in this table mean that further demineralisation occurred in corresponding samples, while "-" figures point to mineral gain thus possible remineralisation

Table 5.14 Depth of crater, μm from sound teeth

Tooth number	Groups							
	Group 1 TO	Group 2 V	Group 3 CK	Group 4 D	Group 5 J	Group 6 S	Group 7 C	Group 8 P
1	6.7	0.0	45.8	13.7	12.8	11.7	7.5	0.0
2	8.7	0.0	39.4	15.1	11.8	13.7	11.5	0.0
3	2.7	0.0	44.2	18.1	14.8	19.5	20.1	0.0
4	5.9	0.0	43.7	18.2	11.9	8.9	10.6	0.0
5	10.0	0.0	32.7	16.5	17.2	8.2	5.9	2.8
6	7.0	0.0	35.5	15.6	10.0	15.3	7.6	1.8
7	8.6	0.0	41.8	14.0	14.1	9.9	11.0	0.4
8	7.8	0.0	49.0	17.6	6.3	14.9	9.3	0.0
9	7.8	0.0	34.7	13.2	8.9	6.9	5.4	0.0
10	5.5	0.0	40.7	21.7	6.1	10.2	5.8	4.4
Mean	**7.1**	**0.0**	**40.8**	**16.4**	**11.4**	**11.9**	**9.5**	**0.9**
SD	**2.0**	**0.0**	**5.2**	**2.6**	**3.6**	**3.9**	**4.4**	**1.6**

Significant difference between all groups, $p < 0.001$ (one-way ANOVA) at the 5% level.

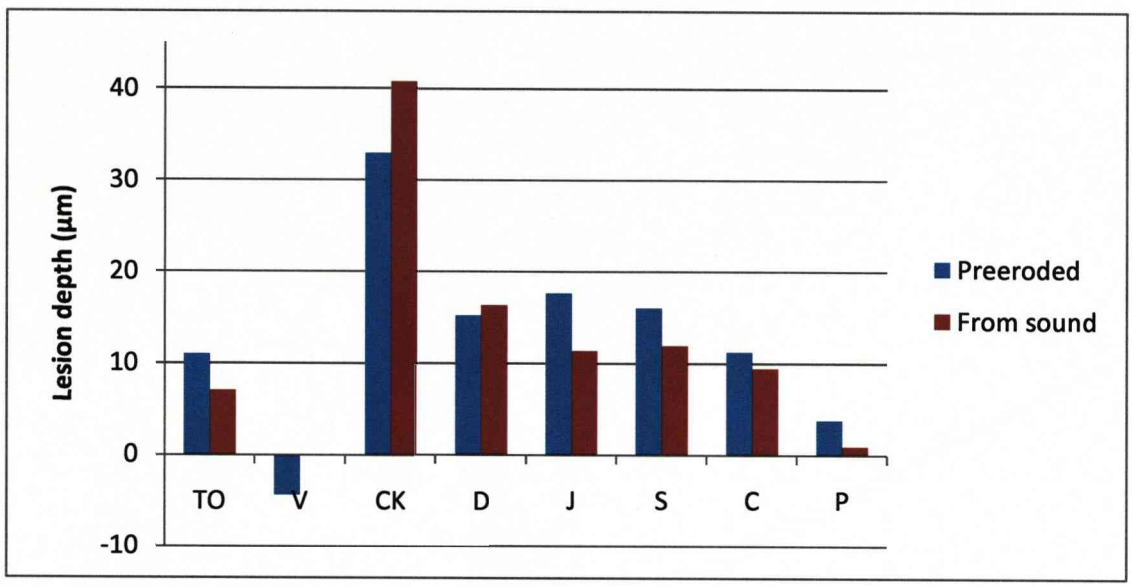


Figure 5.10 Depth of crater, µm

5.5 Discussion

The effect of acid containing soft drinks on human oral health is clear and the situation is deteriorating. The problem of dental erosion attracts considerable attention from health professionals as well as researchers. The increase in soft beverage consumption in the human population at the rate of about 5% per year (Zenith International, 2006) is possibly one of the most important contributing factors to the tendency of increasing prevalence of tooth enamel erosion for the last decades with the most pronounced rate in the younger population (Lussi *et al*, 1991; O'Brien, 1993; Johansson *et al*, 2002; 2003; Jaeggi and Lussi, 2006).

Regarding this aetiological factor of dental erosion several possible preventive measures have been suggested to tackle this growing problem: cutting down the intake of acidic beverages, changing the habit pattern of their consumption and by modifying the formula of drinks reducing their erosive potential (Reussner *et al,* 1975; Grenby, 1996b; Sorvari *et al*, 1988; Sorvari, 1989; Rugg-Gunn *et al*, 1998; Hughes *et al*, 1999b, 2002; Larsen and Nyvad, 1999; West *et al*, 1999, 2003; Moazzez *et al*, 2000; Bartlett *et al*, 2003). Magnesium, fluoride, calcium, phosphate, hydrocolloids, sugar alcohols were used in various sources as additives to alter acidic properties of different carbonated and juice drinks.

In the present study the effect of calcium supplement to commonly used orange drink formula with a brand name of 'Sunny Delight' on sound and pre-

eroded human enamel surfaces was tested in comparison with unmodified branded orange drinks, orange juice prepared from concentrate, sugar containing and sugar-free cola beverages and pH neutral mineral water.

The present study was designed to simulate the physiological situation in the mouth, when common consumption of acidic drinks is introduced indicating so called cycling pattern of demineralisation episodes followed by remineralisation of oral saliva. The tooth samples were dipped three times a day for five minutes in agitated testing solutions, while stored in artificial saliva in between. It is worth noting that these conditions do not fully represent the normal physiological situation observed in the human oral cavity, where several other factors are present, which is believed have an effect on the development of erosive lesion *in vivo*. For instance, abrasive forces of a toothbrush, movements of oral soft tissues like the tongue, cheeks and lips and chewing of hard food produce extra challenge to softened by acidic substances enamel (Davis and Winter, 1980; Bartlett *et al*, 1994; Jaeggi and Lussi, 1999; Attin *et al*, 2001) and may add to the overall rate of enamel demineralisation. Additionally, the presence of the acquired salivary pellicle on tooth surfaces may confer resistance against the erosive influence of acids appearing in the human mouth (Hannig and Balz, 1999; Nekrashevych *et al*, 2004).

The results of the this study demonstrate that there is a significant difference in the erosive potential between tested drinks. The key to the answer, explaining these differences, is more likely to be the variation of composition and

chemical properties of experimental solutions. Titratable acidity, pH, concentration of calcium and phosphates were the parameters that were verified during analysis of the test beverages. Sugar containing cola had the lowest pH (around 3) among acidic drinks used in this study and demonstrated the highest erosive potential under the conditions of this laboratory trial. The highest titratable acidity was recorded in Orange juice followed by all SunnyD drinks, while colas remained at the very bottom of the list with the amount of NaOH used to rise pH to neutral from two to four times lower, than in juice beverages, suggesting that this parameter in isolation could not explain the results of the study.

The main acidic component of cola beverages is phosphoric acid, which is thought to be highly erosive, when compared with organic acids, such as citric, at comparable pH and concentrations (Imfeld, 1983). It is also believed that citric acid is more erosive than predicted in terms of enamel dissolution due to its ability to chelate calcium apatites (McClure and Ruzicka, 1946; Meurman *et al*, 1987) and in some studies comparing commonly available drinks demonstrated higher erosive properties than phosphoric acid (Grenby *et al*, 1989; Attin *et al*, 2005, Jain *et al*, 2007).

It was reported elsewhere that sugar containing beverages are significantly more erosive than their sugar-free counterparts (Grobler *et al*, 1990; Jain *et al*, 2007), which was also confirmed by the results of this study, when demineralisation caused by Coca-Cola and Diet Coke was used in comparison. However, besides the presence of sugar, which is believed to promote the

demineralisation, the differences in pH in these drinks also could contribute to the findings.

Modification of the formula of acidic drinks by adding calcium, phosphate and fluoride in any combinations is being considered as another promising approach to tackle the growing problem of dental erosion. If the drink is supplemented with minerals saturated with respect to apatite it is expected that the dental enamel subjected to such altered drink formulations will not be completly and irreversibly damaged (Larsen and Nyvad, 1999). Several other studies demonstrated the effect of calcium added to acidic beverages in reducing their erosive potential (Beiraghi *et al*, 1989; Larsen and Nyvad, 1999; West *et al*, 2003; Attin *et al*, 2005). The present study revealed that supplying citric acid containing juice drinks with calcium at a concentration of 400 ppm completely prevented tooth enamel of demineralisation. There was no statistical difference between demineralisation caused by Drink P and pH neutral mineral water ($p>0.05$).

Table 5.15 shows the ranking order of drinks utilised during this study in their ability to demineralise human enamel as estimated by TMR of from sound tooth samples. It is clearly illustrated that Coca-Cola caused the most damage to enamel followed by fruit drinks and sugar-free cola, while the negative control (mineral water) and calcium-added juice drink did not initiate any visible erosion, when viewed under TMR. In Table 5.16 shows the tested drinks ranked in order of their pH, titratable acidity and concentration of calcium in relation to their ability to erode enamel. With respect to pH the drinks show exactly the same trend as

described in Table 5.15 with Coca-Cola leading the list of the most erosive substances tested during this study. However, within the fruit drinks the difference between their pH values are small and could not explain the significant difference in their erosive potential as measured by TMR, while the pH is well below critical value of pH 5 and fall in the range between pH 3.44 – 3.83. The amount of titratable acid in beverages varies significantly between them with differences of up to 4 times. The most acidic drinks from this point of view are the Orange juice, Drink C and Drink P, while both colas were placed at the bottom of the list with less significant amount of titratable acid (mineral water does not have any acid).

Table 5.15 The ranking of drinks according their erosive potential measured using TMR

Rank	**Minerl loss (vol%·µm)**		**Lesion depth (µm)**		**Crater depth (µm)**	
	Type of drink	Value	Type of drink	Value	Type of drink	Value
1	Coca-Cola	3991.8	Coca-Cola	54.9	Coca-Cola	40.8
2	Drink J	2306.3	Drink J	53.1	Diet Coke	16.4
3	Drink S	2224.7	Drink S	48.7	Drink S	11.9
4	Drink C	1900.3	Drink C	45.8	Drink J	11.4
5	Diet Coke	1690.4	Diet Coke	27.8	Drink C	9.5
6	Orange juice	922.5	Orange juice	19.7	Orange juice	7.1
7	Drink P	283.3	Volvic water	10.3	Drink P	0.9
8	Volvic water	224.3	Drink P	10.1	Volvic water	0

Table 5.16 The ranking of drinks according their pH, titratable acidity and concentration of calcium and phosphate

Rank	**pH**		**Titratable acid** (mmoles l^{-})		**Calcium (ppm)**		**Phosphate (ppm)**	
	Type of drink	value	Type of drink	value	Type of drink	value	Type of drink	value
1	Coca-Cola	3.06	Drink C	83.0	Drink C	2.52	Volvic water	0.39
2	Diet Coke	3.44	Orange juice	83.0	Coca-Cola	3.04	Drink P	9.86
3	Drink S	3.51	Drink P	74.0	Volvic water	3.28	Drink C	15.33
4	Drink J	3.56	Drink S	66.5	Diet Coke	4.72	Drink S	24.34
5	Orange juice	3.62	Drink J	57.5	Drink J	5.44	Drink J	26.66
6	Drink C	3.71	Diet Coke	24.0	Drink S	9.04	Diet Coke	83.72
7	Drink P	3.83	Coca-Cola	20.3	Orange juice	78.96	Orange juice	103.34
8	Volvic water	7.13	Volvic water	0	Drink P	415.8	Coca-Cola	252.32

With regard to the concentration of calcium the majority of the tested drinks had very low amounts of this ion and ranged between 2 and 9 ppm, while Orange juice and Drink P had considerably higher concentrations – 79 and 415 ppm of calcium respectively. The concentration of phosphates ranged between 0 and 250 ppm with the highest value recorded in both colas and Orange juice and least significant amount in Volvic water and Drink P (0.39 and 9.86 ppm respectfully). Thus, it could be speculated that under the conditions of the above study the presence of high concentrations of calcium even without substantial amount of phosphates plays the major role in prevention of acidic demineralisation of human enamel caused by low pH drinks, while the presence of phosphates on their own could not provide significant protection against dissolution of tooth apatites in these drinks. The antierosive effect of calcium supplements in acidic drinks or citric acid is supported by a number of studies performed *in vitro* and *in situ* and reported by Beiraghi *et al* (1989), Hughes *et al* (1999a,b), Attin *et al* (2003), Jensdottir *et al* (2005).

As half of each tooth was subjected to an artificial erosive challenge before commencement our cycling model, the difference of the progression of erosion by tested drinks between pre-eroded and from sound samples has been carried out. Student t-test comparison demonstrated that there was a significant difference between relative groups in Coca-Cola and Volvic water ($p < 0.01$) and marginally significant ($p = 0.049$) in Drink C for total mineral loss values. In these groups the mineral loss was significantly higher from sound samples, which could be explained by possibly slower rate of hydroxyapatite dissolution of deeper layers of

enamel. It is well known that the very top layer of the enamel usually strengthened by building up fluoride apatites during the lifetime of human teeth (ten Cate, 1997) from external sources of fluoride like fluoridated toothpaste, drinking water, mouthrinses, food and drinks. But during initial preparation of the samples this layer was removed with abrasion exposing the underlying enamel, which is considered less rich with respect to fluoride.

The finding of the present study showed that erosion was being formed in dissimilar patterns, when compared with different erosive agents. TMR data revealed that for some acidic drinks the bulk of the erosive lesion was produced by a total loss of the enamel forming a much deeper crater of erosion, while for other drinks there was a much wider layer of softened surface as demonstrated in Figure 5.7. The exposure to Coca-Cola led to more aggressive demineralisation of enamel, when the total matrix of hydroxyapatites was removed with a rather small layer of surface softening on the bottom of the forming crater. This is illustrated in Table 5.17 and Figure 5.4 and Figure 5.5. It is clearly seen on these graphs that in from sound samples the lesion depth for Coca-Cola is with the same range as for Drinks J, S, and C (Figure 5.5, $p > 0.05$, Tukey multiple comparisons), while total mineral loss (Figure 5.4) differs significantly when comparing these groups ($p < 0.001$, Tukey multiple comparisons). This finding is illustrated in Figure 5.10, where the depth of formed crater is compared between groups ($p < 0.001$, Tukey multiple comparisons) showing that the craters formed in the samples exposed to sugar containing cola are significantly deeper than in samples exposed to other acidic drinks. These data suggest that in those teeth, where the bulk of the erosion

is developed in the softened surface of enamel, the remineralisation of the body of the lesion is possible in that area as the matrix of the enamel containing crystals of calcium hydroxyapatites remained (ten Cate, 1997). The acidic drinks leading to this type of erosive lesions are safer than those, which lead to mostly the total dissolutions of hydroxyapatite crystals making impossible any natural remineralisation.

Table 5.17 The mean percent (± SD) of crater depth from total depth for 6 acidic drinks caused erosion as measured by TMR for from sound samples ($p < 0.001$, ANOVA, $n = 10$)

Orange juice	**Coca-Cola**	**Diet Coke**	**Drink J**	**Drink S**	**Drink C**
35.7 ± 9.8	73.8 ± 4.4	59.1 ± 5.7	20.9 ± 6.0	24.6 ± 7.8	20.3 ± 5.9

In the present investigation the demineralisation of the tooth surface was assessed using the established tools of QLF and TMR. Both techniques allow detection and quantification the mineral changes in tooth enamel.

TMR used in this experiment is an established tool to detect dental erosion and directly quantify the mineral changes in the body of these types of lesions (Amaechi *et al*, 1998a). It allows measurement of the total depth, the depth of

crater, the total volume of mineral loss and the degree of mineral loss or gain on the surface of the softened enamel. This technique is widely used and is considered to be a 'gold standard' in caries and enamel studies across the research community.

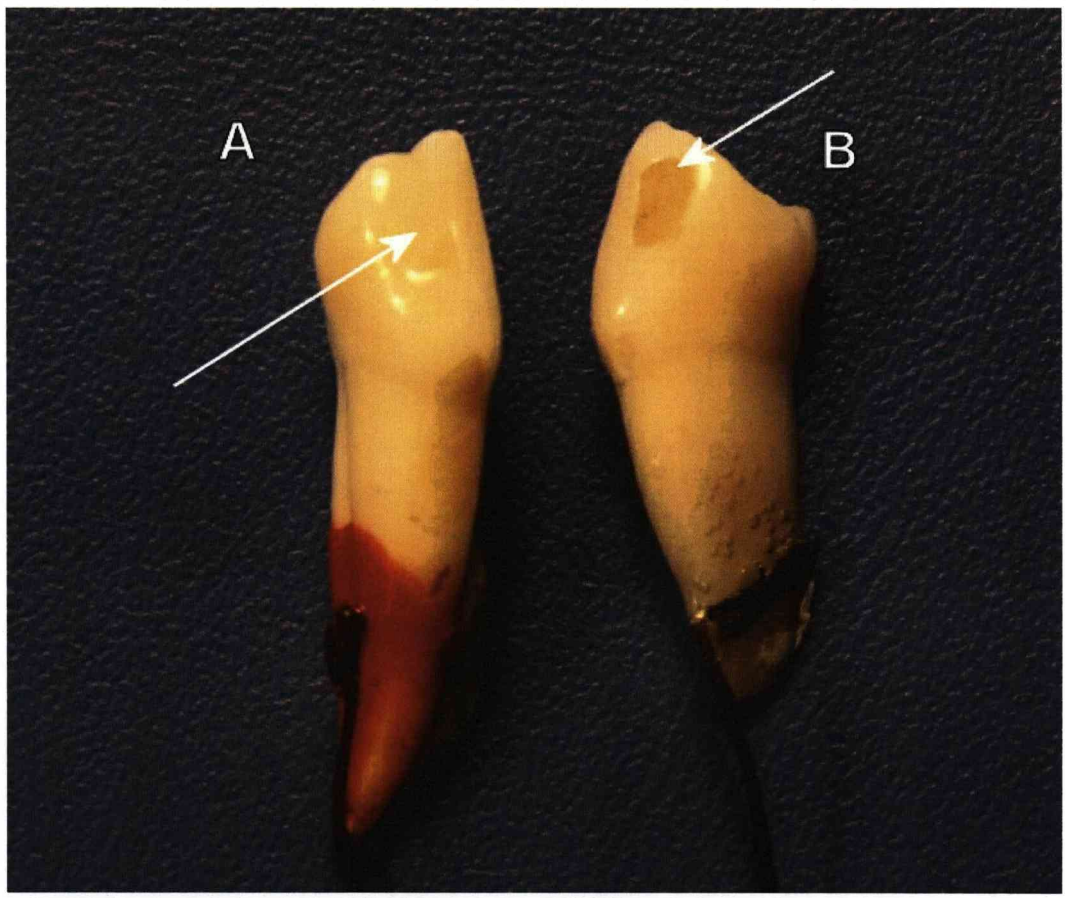


Figure 5.11 The colouring effect of Coca-Cola on the dental enamel. A – tooth exposed to orange juice, B – tooth exposed to Coca-Cola.

QLF as a non-destructive method is useful to monitor the longitudinal changes in caries lesions (Amaechi and Higham, 2002; Pretty *et al*, 2003b) and it was shown in the previous chapters that it could be successfully employed in analysis of erosive lesions. The findings of this study demonstrated that there was a linear correlation between loss of fluorescence and time of erosive challenge for erosive fruit drinks confirming the previous statement. However, initial significant decrease in fluorescence followed by a rise and stabilisation showing no or marginal correlation between fluorescence of demineralised enamel and time of exposure to cola drinks. It could be speculated that this effect was caused by the precipitation of the dye present in these drinks and derived from natural or artificially synthesised extracts. It is believed that this cola dye has changed the colour of the surface of the lesions (Figure 5.11) altering the optical properties of softened enamel and making impossible for QLF to reliably assess the mineral changes taking place during the progression of erosion. The actual erosion in the tooth samples exposed to cola beverages was confirmed by TMR showing advances lesions.

5.6 Conclusion

The findings of the present *in vitro* study showed that out of seven studied acid containing drinks only one (Drink P) did not result in erosive lesions in human teeth under the cycling conditions described above, suggesting that adding calcium to the acidic fruit drink in the form of calcium citrate malate at a concentration of about 400 ppm completely eliminated its erosive potential making it as harmless as mineral water. It was also noted that exposure of human dental enamel to a sugar containing cola beverage (Coca-Cola) led to the most pronounced erosion. In contrast the minor elevation of pH and the use of sugar substitutes significantly reduced the erosive potential of cola drinks (sugar-free Diet Coke).

Moreover, Coca-Cola and Diet Coke caused the most marked erosion from the point of possible remineralisation. The lesions created using these drinks demonstrated visible deeper craters and the shallowest softened demineralised surface layers, while the exposure to acidic fruit drinks created a much wider layer of surface softening which could be restored by means of remineralisation.

It could also be concluded that of the factors considered to be influencing the erosive potential of acidic drinks as pH, titratable acidity, concentration of calcium and phosphate, the most important is probably pH and the presence of calcium ions in high concentrations.

The data of the present investigation showed that Quantitative Light-induced Fluorescence is a sensitive technique, which is appropriate to measure the longitudinal progression of dental erosion caused by citric acid containing fruit juices and drinks, however the use of cola drinks as erosive substances significantly reduce the sensitivity of QLF possibly due to the presence of colours in these drinks, as it affects the optical properties of the demineralised enamel leading to false readings. In contrast, Transverse Microradiography is a useful tool, which can detect erosive lesions and directly measures the amount of minerals in the enamel and dentine in both sound and eroded forms, however this technique is destructive, cannot be used *in vivo* and cannot be used to longitudinally monitor progression and regression of demineralised lesions.

Chapter 6

GENERAL CONCLUSIONS AND SUGGESTIONS FOR FUTURE WORK

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6.1 Conclusions

The studies in this thesis focused on *in vitro* research relating to diagnosis of dental erosion, monitoring progression and developing approaches for prevention and possible early treatment.

The number of existing methods and techniques available to detect and quantify the erosion *in vivo* is limited due to either the destructive nature of the methods or inability to employ them on a natural tooth surface inside the mouth of patient or volunteer. It was shown in this thesis that QLF was capable of accurately detecting dental erosion and longitudinally monitoring its development in terms of further demineralisation or restoration of the softened surface by means of natural saliva or artificially formulated remineralising solutions. It was demonstrated that as the *in vitro* erosive lesion progressed, the degree of tooth fluorescence decreased, revealing good correlation between loss of fluorescence and time of exposure to erosive agents. A significant linear correlation between TMR, a destructive technique commonly used as a gold standard in direct measurement of mineral loss and gain in enamel or dentine exposed to demineralising solutions, and QLF was established, suggesting that by quantifying the changes in tooth fluorescence it is possible to accurately quantify the degree of the progression of dental erosion without destruction of the sample.

The conclusion that not only the increase in the depth of the softened surface but more importantly the depth of crater of erosion plays an important role in the ability of QLF to detect changes in the erosive lesion *in vitro* was found from analysis of the data.

In addition it was noticed that the type of acidic solution used in production of artificial erosive lesions can affect the ability of QLF to correctly reveal and monitor the progression of erosion. While the exposure of teeth to orange juice demonstrated the steady increase in mineral loss, measured by TMR, and fluorescence loss, measured by QLF, the accuracy of the detection of demineralisation in samples exposed to cola drinks using QLF was less pronounced. This finding was explained by the fact that cola drinks have a dark colour and the dye responsible changes the optical properties of the enamel thus affecting the ability of QLF to accurately reveal the demineralisation of enamel.

Due to the complexity of dental erosion seen in clinical situations, where it is virtually impossible to find sharp crater edges and when such phenomena as attrition and abrasion usually take place, the use of QLF technique could be limited and further investigations are required to verify the importance and benefits of QLF *in vivo* studies. It is possible to conclude, however, that QLF is a useful method to study dental erosion using *in vitro* and *in situ* models.

It has been shown that demineralisation taking place during dental erosion can be arrested and in early stages even reversed, when softened surface of enamel is rehardened by incorporation of calcium, phosphates and fluoride into the

weakened matrix of hydroxyapatite crystals. The studies in this thesis have suggested that modification to the formula of remineralising solutions by differing the concentrations of sodium fluoride and calcium lactate, may have the ability to boost their remineralising potential *in vitro*. The results demonstrated that the presence of calcium ions in all concentrations tested not only prevented further destruction of the weakened enamel layer but also led to a noticeable remineralisation of previously softened tooth surfaces, while the absence of this ion did cause such an effect even with the presence of fluoride. However, there was found to be no significant difference between all tested concentrations of calcium in the ability to restore the eroded enamel, suggesting that calcium is possibly the obligatory compound for remineralising solutions designed for treatment of dental erosion and exhibits its desired effect even with small concentrations.

It was also confirmed that adding fluoride to remineralising solutions slightly increases its remineralising capability; however, a dose dependent effect of fluoride was observed, when high (more than 500 ppm) concentrations of this ion led to further demineralisation of lesions in agitated conditions.

The recommended formula of remineralising solution considered for prevention and treatment of dental erosion could be beneficial for those patients, who suffer from frequent episodes of vomiting (bulimia/anorexia nervosa), have severe symptomatic regurgitation and gastroesophageal reflux, when the use of toothbrush directly after such episodes can cause accelerated abrasion of softened

tooth surface and in contrary, the use of remineralising solutions would possibly restore this weakened superficial enamel layer.

Another approach in prevention of dental erosion focusing on the reduction of the erosive potential of acidic drinks was also investigated. The addition of relatively high concentration of calcium in the form of calcium citrate malate into highly acidic fruit drinks controlled its ability to erode human enamel in a cycling model study *in vitro*. The use of calcium citrate malate was dictated by the properties of this type of calcium salt. Being easily dissolved in water it releases calcium ions and a calcium citrate complex, which has been shown to protect dental enamel against acidic dissolution and does not alter the organoleptic properties of the drink demonstrating high bioavailability.

The use of sugar substitutes and minor increases in the pH of acidic beverages containing phosphoric acid caused reduction in mineral loss in exposed human enamel in the study, with sugar-free cola drink exhibiting significantly less erosive potential than its sugar containing counterpart. Bearing in mind the worldwide tendency to increase the consumption of non-alcoholic drinks, which is believed to cause the rise of the prevalence of dental erosion, the modification of composition of acidic soft drinks from the point of their erosiveness could be a promising preventive regime.

It is interesting to note an observation relating to the comparative properties of human and bovine teeth and their ability to develop erosion *in vitro* and *in situ*. The results showed that teeth collected from the cattle are more prone

to the acidic dissolution probably due to the degree of their porosity and mineralisation; however, it has been demonstrated that pattern of the erosive demineralisation, detection of erosion, quantification using the available methods similar to that of human teeth. Thus, bovine teeth could be an appropriate alternative to the use of human teeth. This is likely to be of importance especially if, as predicted, the availability of human teeth becomes limited.

6.2 Suggestions for future studies

The studies reported in this thesis have shown that artificially produced erosive lesions can be remineralised *in vitro* using remineralising solution containing adequate concentrations of calcium and fluoride. Early corresponding reports and epidemiological studies suggest that, while the problem of dental erosion is deteriorating in the human population, it is worth proposing development of active prevention and treatment strategies dealing with early manifestations of this condition. Thus it may be necessary to conduct in future an *in vivo* or an *in situ* clinical study to confirm the antierosive properties of the remineralising solution. *In vitro* work with this solution could concentrate on improving its remineralising potential focusing on the most effective concentration of fluoride in the range between 500 and 1000 ppm.

Another factor, which could be addressed in future, is the effect of toothbrush abrasion on softened enamel by erosive challenge, and determination of the minimum time within which the remineralising solution is able to restore the natural resistance of enamel to abrasive forces of a toothbrush or other factors. Laboratory studies employing toothbrushing procedures combined with artificial erosion could possibly help better understanding of the mechanism of detection of dental erosion by QLF, when the softened superficial layer of eroded enamel is partially or completely removed by toothbrushing.

As was verified, that addition of calcium into an acidic fruit drink such as Sunny D lead to significant reduction of erosive potential; thus, it would be worthwhile to confirm antierosive properties of this drink in a clinical trial as well as to investigate any potential cariogenic effect of this sugar containing drink. Concentration of calcium in the dental plaque, pH of the plaque would be the key issues to address in caries related studies. Another aspect to be clarified would be studies to establish the interaction of factors thought to affect the erosive potential of acidic beverages such as their pH and presence/or concentrations of fermented sugars.

With the advances of science and emergence of new evaluation techniques further progress in studying dental health conditions will occur. Two new optical imaging methods may be used in future application for evaluation of dental erosion. One is Optical Coherence Tomography (OCT), a technique built around the principles of a confocal microscopy and low coherence interferometry, where high resolution longitudinal (B-scans) and en-face (C-scans) images and depth resolved reflectivity scans (A-scans) of dental tissues are produced. OCT was tested in studying carious lesion and the data was correlated to TMR and QLF (Amaechi *et al*, 2001, 2002, 2003a,b, 2004). A short pilot study to examine the prospects to image dental erosion lesions was performed and the possibility to use OCT in erosion studies was established (Figure 6.1).

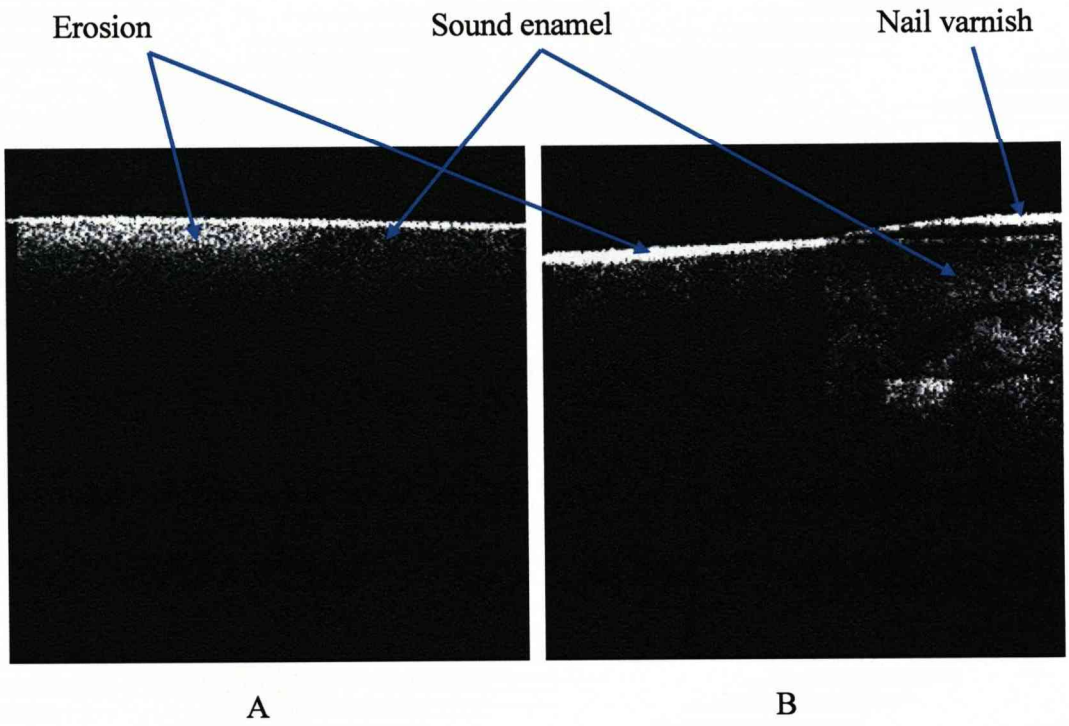
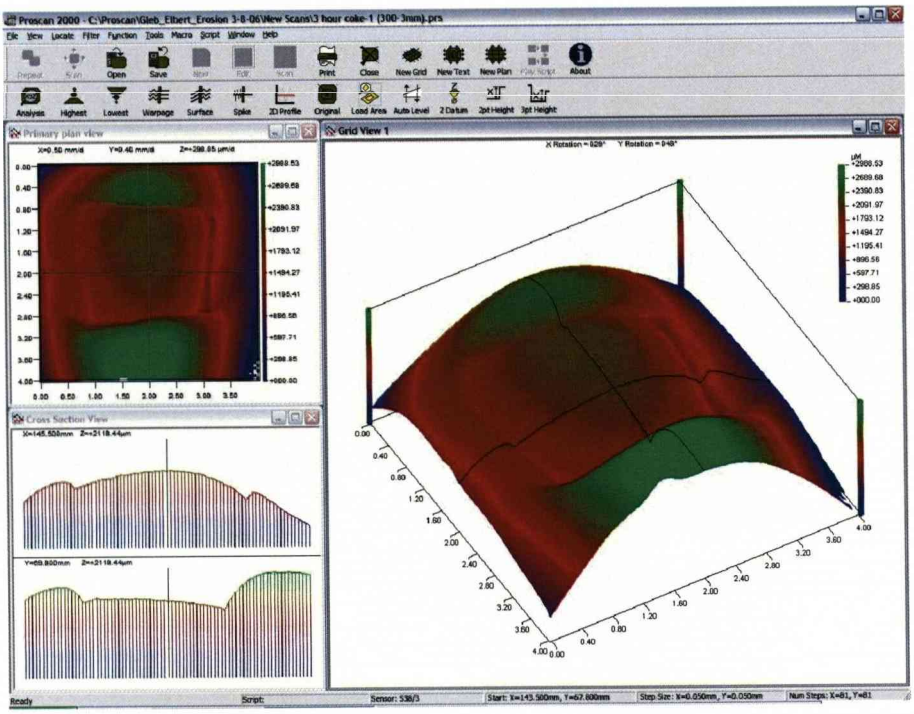
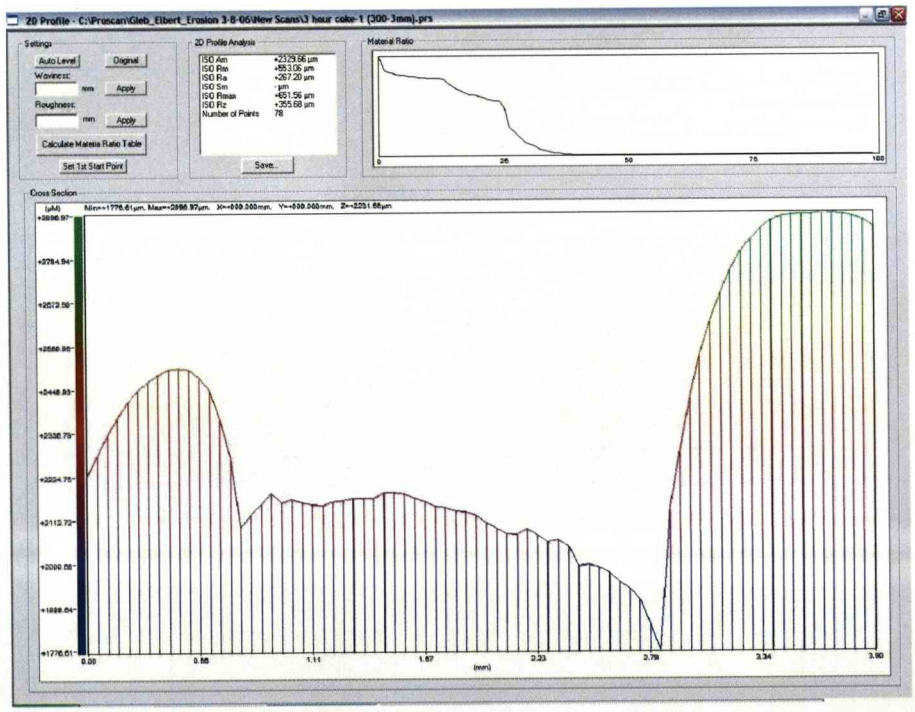


Figure 6.1 Longutudinal OCT images (B-scans) of artificially produced dental erosion, A – without nail varnish above sound enamel, B – with nail varnish above sound enamel

Another method to study dental erosion is available using laser profilometry (e.g. Proscan 2000, Scantron Industrial Products Ltd, Taunton, UK). It is based on the innovative optical principle of chromatic confocal imagery, when a range of non-contact dimensional, high-resolution sensors are used for microtopography and 3-dimentional imaging of tooth surface with a resolution as low as 5nm. Artificially produced erosive lesions were scanned during a pilot study with this tool producing high quality pictures, where the topography of lesions could be quantified (Figure 6.2).



A



B

Figure 6.2 Proscan imaging analysis software package demonstrating high-resolution A – 3D view and B – 2D profiles of erosive lesion

It is envisaged that if these non-destructive methods could be combined it offers new approaches for the study of dental erosion *in vitro* as well as *in vivo* or *in situ*.

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APPENDIX

The Remineralisation Potential of Artificial Saliva with Different Concentrations of Calcium on Eroded Lesion *in-vitro*

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In the presence of calcium and phosphate artificial saliva exhibits its remineralising properties on early enamel lesions. On the other hand, excessive concentration of these ions could lead to rehardening of only the superficially softened layer of the lesion thus blocking access to the subsurface space and leaving it hypomineralised. The aim of this study was to assess the difference in the remineralising potential of artificial saliva with different concentrations of calcium on dental erosion. Freshly extracted human molars were coated with acid-resistant nail varnish except for a rectangular window on buccal surface of the tooth. Artificial eroded lesions were produced by immersing the samples in agitated orange juice (pH 3.6) for 90 minutes. Two slabs and control sections were cut from each molar and randomly allocated into 7 groups with 9 slabs in each. The specimens were exposed for 28 days at 37°C in artificial saliva with fixed concentrations of phosphate (100ppm) and fluoride (0.05ppm) and calcium at either 0, 50, 100, 250, 500 or 1000 ppm (in form of calcium lactate); natural centrifuged saliva was chosen as positive control. All solutions were changed daily. The amount of mineral loss (ΔZ) and lesion depth (ld) were measured using microradiography and the data analysed by paired t-test and one-way ANOVA (α=0.05). Natural saliva and all remineralising solutions with added calcium demonstrated protective abilities on eroded enamel in comparison with 0 ppm calcium group ($p<0.0001$). The most effective remineralising effect was observed in the group with 250 ppm of calcium, however the difference between all calcium-containing groups was not significant ($p>0.05$). It was concluded that artificial saliva containing calcium has a protective and remineralising effect on eroded enamel lesion *in vitro*.

Remineralising Effect of Fluoride on Enamel Erosion *in vitro*

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The effect of different levels of fluoride on the remineralisation of dental erosion was investigated *in vitro*. Freshly extracted human molars were coated with acid-resistant nail varnish except for a rectangular window on buccal surface of the tooth. Eroded enamel lesions were produced by immersing the samples in agitated orange juice (pH 3.6) for 90 minutes. Three slabs and control sections were cut from each molar and randomly allocated into 7 groups with 12 slabs in each. The specimens were exposed for 28 days at 37°C to artificial saliva with fixed concentrations of calcium lactate (250 ppm Ca2+) and phosphate (100 ppm) and fluoride at either 0, 100, 250, 500, 1000 or 12,000 ppm. Centrifuged natural saliva with the fluoride concentration adjusted to 0.05 ppm was used as positive control. All solutions were changed daily. The amount of mineral loss (ΔZ) and lesion depth (ld) were measured using microradiography and the data analysed by paired t-test, one-way ANOVA and multiple comparison ($\alpha=0.05$). Exposure to natural saliva and remineralising solutions with 100, 250 and 500 ppm of fluoride resulted in an increase of mineral content of the lesions, while in the groups with 0, 1000 and 12,000 ppm of fluoride further mineral loss was found with the maximum in the 1000 ppm group ($p<0.0001$). The greatest remineralisation was observed in the group with 500 ppm of fluoride, however multiple comparisons demonstrated that the difference between the groups with mineral gain was not significant ($p>0.05$). It was concluded that artificial saliva containing calcium, phosphate and up to 500 ppm fluoride has a protective and remineralising effect on eroded enamel lesion *in vitro*.

Quantitative Light-Induced Fluorescence Correlated with Transverse Microradiography for Quantification of Dental Erosion *in vitro*

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The ability of Quantitative Light-Induced Fluorescence (QLF) to detect and quantitatively monitor dental caries is well established. The aim of the present study was to determine the ability of QLF to detect and quantify dental erosion, and correlate it with that of an established method of quantifying erosion, transverse microradiography (TMR). Bovine incisors were cleaned with wet pumice and each tooth was cut into two halves. Each half was coated with non-fluorescent acid-resistant nail varnish except for an exposed window. Erosion was created by immersing the teeth in agitated orange juice (pH 3.5) at room temperature. Erosion was measured in one half of each tooth with QLF and in the second half with TMR as follows. Prior to erosion, QLF image was taken from one half of each tooth while an enamel section was cut from the second half. This procedure was repeated every 30 minutes during the erosive attack for a period of 300 minutes. The images and enamel sections were analysed for quantification of mineral loss using QLF systems (ΔQ) and TMR (ΔZ) respectively. The mineral loss, measured by both systems, increased with increasing erosion time. A linear correlation (Pearson correlation coefficient, $r = 0.98$) was observed between ΔQ (range 11.08 – 88.4 $mm^2 \cdot \%$) measured by QLF and ΔZ (range 909.8 – 6711.5 $vol\% \cdot \mu m$), measured by TMR. It was concluded that QLF was able to detect and monitor mineral loss due to erosion, and could be a useful tool for assessment of mineral loss following erosive challenges. Although this study was performed *in vitro*, it is envisaged that this technique will also have an application for the *in vivo* assessment of dental erosion.

Measurement of Dental Erosion Using Quantitative Light-Induced Fluorescence

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The use of Quantitative Light-Induced Fluorescence (QLF) to detect and quantitatively monitor dental caries is now well established, and more recent studies suggest its possible ability to measure dental erosion. **Objective**: To investigate the ability of QLF to detect and quantify dental erosion, and correlate it with transverse microradiography (TMR). **Methods**: 13 bovine incisors and 13 human molars were prepared and each tooth was cut into two halves. The slabs were coated with non-fluorescent acid-resistant nail varnish except for an exposed window on buccal surface. Erosion was created by immersing the teeth in agitated orange juice (pH 3.5) at room temperature. Erosion was quantified in one half of each tooth with QLF and in the second half with TMR as follows. After initial 30 minutes (60 minutes for human teeth) of erosive challenge, QLF image was taken from one half of each tooth and fluorescence loss (ΔQ) quantified, while an enamel section was cut from the second half and microradiographed. This procedure was repeated every 30 minutes for a total period of 300 minutes. From microradiographs the depth of the crater (CD) created by erosion, and the mineral loss, first for the entire eroded lesion (ΔZ_e) and then for only the demineralised tissue at the lesion base (ΔZ_b) were determined. **Results:** Squared Pearson correlation coefficient (r^2) indicated a linear correlation between ΔQ and ΔZ_e in both bovine teeth (r^2=0.98; regression equation, $\Delta Q = 0.83 + 0.01\Delta Z$), and human teeth ($r^2$=0.99; $\Delta Q = -3.52 + 0.01\Delta Z$). A stronger correlation was observed between ΔQ and CD [r^2=0.99 (bovine); r^2=0.96 (human)], than between ΔQ and ΔZ_b [r^2=0.81 (bovine); r^2=0.79 (human)]. **Conclusion:** QLF was able to monitor the mineral lost during erosive challenge, due mainly to its ability to measure the change in depth of the crater formed during the erosive attack.

Erosive Potential of Soft Drinks

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Objectives: The aim of this study was to evaluate the erosive potential of soft drinks on human enamel *in vitro* using Transverse Microradiography (TMR) and Quantitative Light-induced Fluorescence (QLF).
Methods: Forty human premolars painted with an acid resistant nail varnish except for a buccal surface window were stored for four weeks in artificial saliva. The samples were divided into four groups and subjected to testing solutions 3 times a day for 5 minutes during weekdays. Erosive properties of orange juice (Tesco value), cola (Coca-Cola) and sugar-free cola (Diet Coke) were tested against a negative control mineral water (Volvic). Twice a week the samples were photographed using QLF and at the end of the experiment TMR analysis was performed.
Results: With QLF there was a significant correlation between exposure time and fluorescence loss (ΔQ) for orange juice ($r = 0.93$, $p < 0.001$) and sugar-free cola ($r = 0.68$, $p = 0.043$), while cola and water groups had no significant correlation ($r = 0.24$, $p > 0.05$). Direct measurement of mineral loss using TMR demonstrated that all three acid containing drinks developed artificial erosive lesions. Mineral loss (ΔZ) for cola was 3991.8 ± 434.6, for sugar-free cola – 1690.4 ± 257.1, for orange juice – 922.5 ± 237.0 and mean delta Z value recorded on the surface of teeth exposed to mineral water was 224.3 ± 67.0 with significant difference between all groups (ANOVA and Tukey post hoc, $p < 0.001$). Mean depth of the crater of the lesion as a percentage of the whole lesion depth was 36.1, 74.1 and 59.3% for orange juice, cola and sugar-free cola respectively (ANOVA and Tukey post hoc, $p < 0.001$).
Conclusion: Among all three acid containing drinks, cola had the greatest erosive potential. Using sugar substitutes significantly reduced the erosive properties of carbonated cola.